

diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (3 mL) and cooled to 0°C .

Triethylamine (0.27 mL, 1.92 mmol) was added followed by the treatment of

- 5 benzenesulfonyl chloride (84 mg, 0.48 mmol). The solution was stirred for 30 min at 0°C and then warmed to room temperature for 30 min. The product was partitioned between CH_2Cl_2 and 0.2 N HCl. The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the
- 10 monophospholactate (0.33 g, 85%, GS 192779, 1:1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 7.78 (dd, 2H), 7.59 (m, 3H), 7.38-7.18 (m, 7H), 6.93 (dd, 2H), 5.66 (m, 1H), 5.18-4.93 (m, 3H), 4.56-4.4 (m, 2H), 4.2 (m, 2H), 4.1-3.7 (m, 6H), 3.17 (m, 1H), 3.02-2.8 (m, 6H), 1.84 (m, 1H), 1.82-1.5 (m, 5H), 1.27 (m, 3H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.88 (d, $J = 6.3$ Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.4, 15.3.

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Example 26

Monophospholactate 27: A solution of 25 (0.50 g, 0.64 mmol) in CH_2Cl_2 (1.0 mL) at 0°C was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted

20 with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (4 mL) and cooled to 0°C . Triethylamine (0.36 mL, 2.56 mmol) was added followed by the treatment of 4-fluorobenzenesulfonyl chloride (0.13 g, 0.64 mmol). The solution was stirred for 30 min at 0°C and then warmed to room

25 temperature for 30 min. The product was partitioned between CH_2Cl_2 and 0.2 N HCl. The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.44 g, 81%, GS 192776, 3/2 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 7.80 (m, 2H), 7.38-7.15 (m,

30 9H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-4.9 (m, 3H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.1-3.7 (m, 6H), 3.6 (broad, s, 1H), 3.17 (m, 1H), 3.02-2.75 (m, 6H), 1.85 (m, 1H), 1.7-1.5 (m, 5H), 1.26 (m, 3H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.88 (d, $J = 6.3$ Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.3, 15.2.

Example 27

Monophospholactate 28: A solution of 25 (0.50 g, 0.64 mmol) in CH_2Cl_2 (1.0 mL) at 0°C was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (3 mL) and cooled to 0°C . Triethylamine (0.45 mL, 3.20 mmol) was added followed by the treatment of hydrogen chloride salt of 3-pyridinylsulfonyl chloride (0.14 g, 0.65 mmol). The solution was stirred for 30 min at 0°C and then warmed to room temperature for 30 min. The product was partitioned between CH_2Cl_2 and H_2O . The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.41 g, 79%, GS 273806, 1:1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 9.0 (s, 1H), 8.83 (dd, 1H), 8.06 (d, $J = 7.8$ Hz, 1H), 7.5 (m, 1H), 7.38-7.15 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.18-4.95 (m, 3H), 4.6-4.41 (m, 2H), 4.2 (m, 2H), 4.0 (m, 1H), 3.95-3.76 (m, 6H), 3.23-2.8 (m, 7H), 1.88 (m, 1H), 1.7-1.5 (m, 5H), 1.26 (m, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 0.83 (d, $J = 6.6$ Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.3, 15.3.

20 Example 28

Monophospholactate 29: A solution of compound 28 (0.82 g, 1.00 mmol) in CH_2Cl_2 (8 mL) at 0°C was treated with *m*CPBA (1.25 eq). The solution was stirred for 1 h at 0°C and then warmed to room temperature for an additional 6 h. The reaction mixture was partitioned between CH_2Cl_2 and saturated NaHCO_3 . The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.59 g, 70%, GS 273851, 1:1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 8.63 (dd, 1H), 8.3 (dd, 1H), 7.57 (m, 1H), 7.44 (m, 1H), 7.38-7.13 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-5.05 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.0-3.73 (m, 6H), 3.2 (m, 2H), 3.0 (m, 4H), 2.77 (m, 1H), 1.92 (m, 1H), 1.7-1.49 (m, 5H), 1.26 (m, 3H), 0.91 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.3, 15.3.

Example 29

Monophospholactate 30: A solution of compound 28 (71 mg, 0.087 mmol) in CHCl_3 (1 mL) was treated with MeOTf (18 mg, 0.11 mmol). The solution was stirred at room temperature for 1 h. The reaction mixture was concentrated and co-evaporated with toluene (2 x), CHCl_3 (2 x) and dried under vacuum to give the monophospholactate (81 mg, 95%, GS 273813, 1:1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 9.0 (dd, 1H), 8.76 (m, 2H), 8.1 (m, 1H), 7.35-7.1 (m, 7H), 6.89 (m, 2H), 5.64 (m, 1H), 5.25-5.0 (m, 3H), 4.6-4.41 (m, 5H), 4.2 (m, 2H), 3.92-3.72 (m, 6H), 3.28 (m, 2H), 3.04-2.85 (m, 3H), 2.62 (m, 1H), 1.97 (m, 1H), 1.62-1.5 (m, 5H), 1.25 (m, 3H), 0.97 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.4, 15.4.

10 Example 30

Dibenzylphosphonate 31: A solution of compound 16 (0.15 g, 0.18 mmol) in CHCl_3 (2 mL) was treated with MeOTf (37 mg, 0.23 mmol). The solution was stirred at room temperature for 2 h. The reaction mixture was concentrated and co-evaporated with toluene (2 x), CHCl_3 (2 x) and dried under vacuum to give the dibenzylphosphonate (0.17 g, 95%, GS 273812) as a white solid: ^1H NMR (CDCl_3) δ 9.0 (dd, 1H), 8.73 (m, 2H), 8.09 (m, 1H), 7.35 (m, 10H), 7.09 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.1 Hz, 2H), 5.61 (d, J = 4.2 Hz, 1H), 5.2-4.96 (m, 6H), 4.54 (s, 3H), 4.2 (dd, 2H), 3.92-3.69 (m, 6H), 3.3 (m, 2H), 3.04-2.6 (m, 5H), 1.97 (m, 1H), 1.6 (m, 2H), 0.98 (m, 6H); ^{31}P NMR (CDCl_3) δ 20.4.

20 Example 31

Dibenzylphosphonate 32: A solution of compound 16 (0.15 g, 0.18 mmol) in CH_2Cl_2 (3 mL) at 0°C was treated with *m*CPBA (1.25 eq). The solution was stirred for 1 h at 0°C and then warmed to room temperature overnight. The reaction mixture was partitioned between 10% 2-propanol/ CH_2Cl_2 and saturated NaHCO_3 . The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ CH_2Cl_2) to give the dibenzylphosphonate (0.11 g, 70%, GS 277774) as a white solid: ^1H NMR (CDCl_3) δ 8.64 (m, 1H), 8.27 (d, J = 6.9 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.36 (m, 11H), 7.10 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.22-5.02 (m, 6H), 4.21 (dd, 2H), 3.99-3.65 (m, 6H), 3.2 (m, 2H), 3.03-2.73 (m, 5H), 1.90 (m, 1H), 1.66-1.56 (m, 2H), 0.91 (m, 6H); ^{31}P NMR (CDCl_3) δ 20.3.

Example 32

Phosphonic Acid 33: To a solution of dibenzylphosphonate 32 (0.1 g, 0.12 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 1 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and purified by HPLC to give the phosphonic acid (17 mg, GS 277775) as a white solid: ¹H NMR (CD₃OD) δ 8.68 (s, 1H), 8.47 (d, J = 6.0 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.68 (m, 1H), 7.14 (m, 2H), 6.90 (d, J = 7.8 Hz, 2H), 5.58 (d, J = 5.4 Hz, 1H), 5.00 (m, 1H), 4.08 (d, J = 9.9 Hz, 2H), 3.93-3.69 (m, 6H), 3.4-2.9 (m, 7H), 2.5 (m, 1H), 2.04 (m, 1H), 1.6-1.35 (m, 2H), 0.92 (m, 6H); ³¹P NMR (CD₃OD) δ 15.8.

Example 33

Monophospholactate 34: A solution of 25 (2.50 g, 3.21 mmol) in CH₂Cl₂ (5.0 mL) at 0°C was treated with trifluoroacetic acid (2.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH₂Cl₂ (30 mL) and cooled to 0°C. Triethylamine (1.79 mL, 12.84 mmol) was added followed by the treatment of 4-formylbenzenesulfonyl chloride (0.72 g, 3.53 mmol) and the solution was stirred at 0°C for 1 h. The product was partitioned between CH₂Cl₂ and 5% HCl. The organic phase was washed with H₂O, saturated NaCl, dried with Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the monophospholactate (2.11 g, 77%, GS 278052, 1:1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 10.12 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 7.5 Hz, 2H), 7.38-7.15 (m, 7H), 6.94 (m, 2H), 5.67 (m, 1H), 5.18-4.91 (m, 3H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.0-3.69 (m, 6H), 3.57 (broad, s, 1H), 3.19-2.8 (m, 7H), 1.87 (m, 1H), 1.69-1.48 (m, 5H), 1.25 (m, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); ³¹P NMR (CDCl₃) δ 17.3, 15.2.

Example 34

Monophospholactate 35: A solution of 34 (0.60 g, 0.71 mmol) and morpholine (0.31 mL, 3.54 mmol) in EtOAc (8 mL) was treated with HOAc (0.16 mL, 2.83 mmol) and NaBH₃CN (89 mg, 1.42 mmol). The reaction mixture was stirred at room temperature for 4 h. The

product was partitioned between EtOAc and H₂O. The organic phase was washed with brine, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (6% 2-propanol/CH₂Cl₂) to give the monophospholactate (0.46 g, 70%, GS 278115, 1:1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 7.74 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.38-7.15 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-5.0 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 3.97-3.57 (m, 12H), 3.2-2.78 (m, 7H), 2.46 (broad, s, 4H), 1.87 (m, 1H), 1.64-1.5 (m, 5H), 1.25 (m, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); ³¹P NMR (CDCl₃) δ 17.3, 15.3.

10 Example 35

Monophospholactate 37: A solution of 25 (0.50 g, 0.64 mmol) in CH₂Cl₂ (2.0 mL) at 0°C was treated with trifluoroacetic acid (1 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH₂Cl₂ (3 mL) and cooled to 0°C. Triethylamine (0.45 mL, 3.20 mmol) was added followed by the treatment of 4-benzyloxybenzenesulfonyl chloride (0.18 g, 0.64 mmol, prepared according to Toja, E. et al. Eur. J. Med. Chem. 1991, 26, 403). The solution was stirred for 30 min at 0°C and then warmed to room temperature for 30 min. The product was partitioned between CH₂Cl₂ and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/CH₂Cl₂) to give the monophospholactate (0.51 g, 85%) as a white solid.

25 Example 36

Monophospholactate 38: To a solution of 37 (0.48 g, 0.52 mmol) in EtOH (15 mL) was added 10% Pd/C (0.10 g). The suspension was stirred under H₂ atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and the crude product was purified by column chromatography on silica gel (5% 2-propanol/CH₂Cl₂) to give the monophospholactate (0.38 g, 88%, GS 273838, 1:1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 8.86 (dd, 1H), 7.42-7.25 (m, 9H), 6.91 (m, 4H), 5.73 (d, J = 5.1 Hz, 1H), 5.42 (m, 1H), 5.18 (m, 2H), 4.76-4.31 (m, 2H), 4.22 (m, 2H), 4.12-3.75 (m, 6H), 3.63 (broad, s, 1H), 3.13 (m, 3H), 2.87 (m, 1H), 2.63

(m, 1H), 2.4 (m, 1H), 2.05 (m, 2H), 1.9 (m, 1H), 1.8(m, 1H), 1.6 (m, 3H), 1.25 (m, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.1, 15.7.

Example 37

- 5 Monophospholactate 40: A solution of 25 (0.75 g, 0.96 mmol) in CH_2Cl_2 (2.0 mL) at 0°C was treated with trifluoroacetic acid (1 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt
- 10 which was dissolved in CH_2Cl_2 (4 mL) and cooled to 0°C . Triethylamine (0.67 mL, 4.80 mmol) was added followed by the treatment of 4-(4'-benzyloxycarbonyl piperazinyl)benzenesulfonyl chloride (0.48 g, 1.22 mmol, prepared according to Toja, E. et al. *Arzneim. Forsch.* 1994, 44, 501). The solution was stirred at 0°C for 1 h and then warmed to room temperature for 30 min. The product was partitioned between 10% 2-
- 15 propanol/ CH_2Cl_2 and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.63 g, 60%) as a white solid.

20 Example 38

- Monophospholactate 41: To a solution of 40 (0.62 g, 0.60 mmol) in MeOH (8 mL) and EtOAc (2 mL) was added 10% Pd/C (0.20 g). The suspension was stirred under H_2 atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was treated with 1.2 equivalent of TFA, co-evaporated
- 25 with CHCl_3 and dried under vacuum to give the monophospholactate (0.55 g, 90%) as a white solid.

Example 39

- Monophospholactate 42: A solution of 41 (0.54 g, 0.53 mmol) and formaldehyde (0.16 mL, 5.30 mmol) in EtOAc (10 mL) was treated with HOAc (0.30 mL, 5.30 mmol) and NaBH_3CN (0.33 g, 5.30 mmol). The reaction mixture was stirred at room temperature overnight. The product was partitioned between EtOAc and H_2O . The organic phase was washed with brine, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column
- 30

chromatography on silica gel (6% 2-propanol/CH₂Cl₂) to give the monophospholactate (97.2 mg, 20%, GS 277937, 1:1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 7.64 (d, J = 9.0 Hz, 2H), 7.38-7.17 (m, 7H), 6.95-6.88 (m, 4H), 5.67 (m, 1H), 5.2-4.96 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 3.97-3.64 (m, 8H), 3.49-3.37 (m, 4H), 3.05-2.78 (m, 12H), 1.88-1.62 (m, 3H), 1.58 (m, 3H), 1.25 (m, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); ³¹P NMR (CDCl₃) δ 17.3, 15.3.

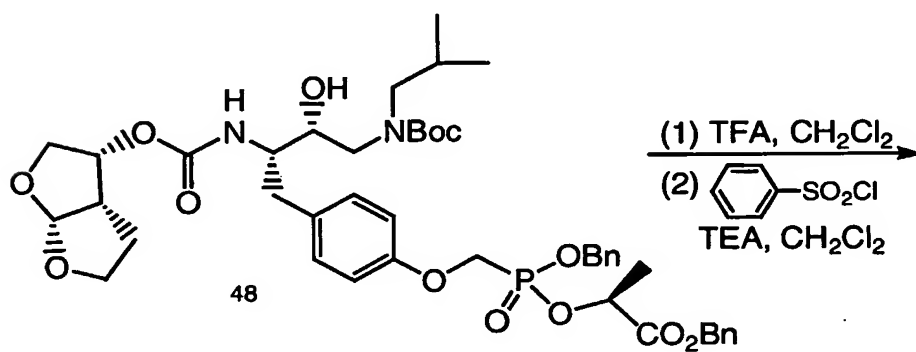
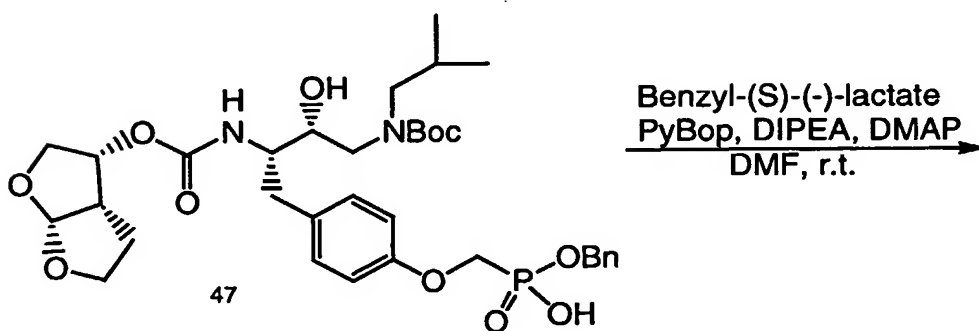
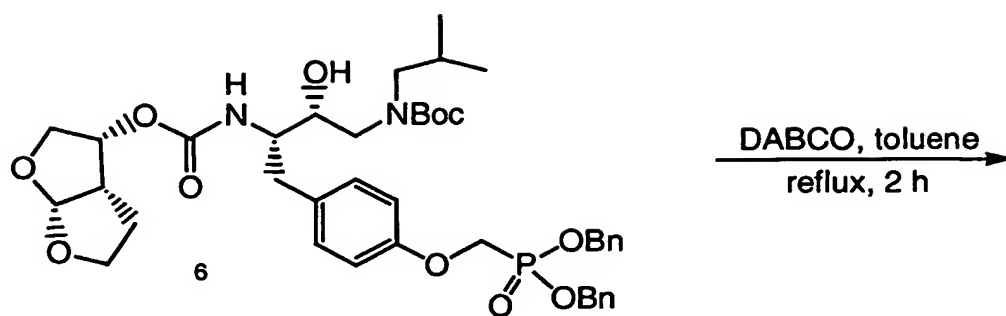
Example 40

Monophospholactate 45: A solution of 43 (0.12 g, 0.16 mmol) and lactate 44 (0.22 g, 1.02 mmol) in pyridine (1 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.17 g, 0.83 mmol) was added. The reaction mixture was stirred at 70°C for 4 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H₂O, saturated NaCl, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the monophospholactate (45 mg, 26%) as a white solid.

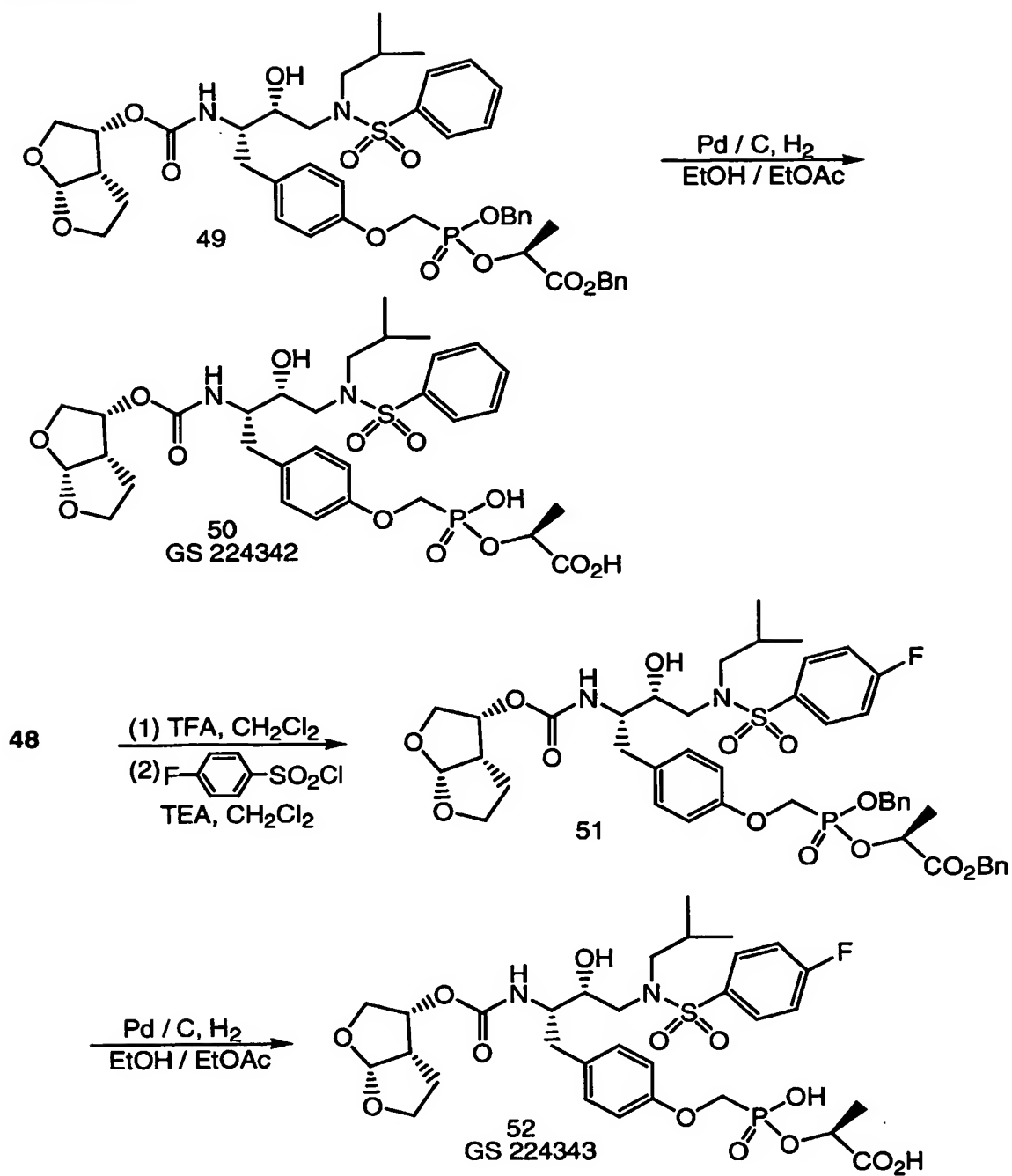
Example 41

Alcohol 46: To a solution of 45 (40 mg, 0.042 mmol) in EtOAc (2 mL) was added 20% Pd(OH)₂/C (10 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 3 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and the product was dried under vacuum to give the alcohol (33 mg, 90%, GS 278809, 3/2 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 7.72 (d, J = 8.7 Hz, 2H), 7.39-7.15 (m, 7H), 7.02-6.88 (m, 4H), 5.66 (d, J = 4.5 Hz, 1H), 5.13-5.02 (m, 2H), 4.54-4.10 (m, 4H), 4.00-3.69 (m, 11H), 3.14 (m, 1H), 3.02-2.77 (m, 6H), 1.85-1.6 (m, 6H), 0.94 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H); ³¹P NMR (CDCl₃) δ 17.4, 15.9.

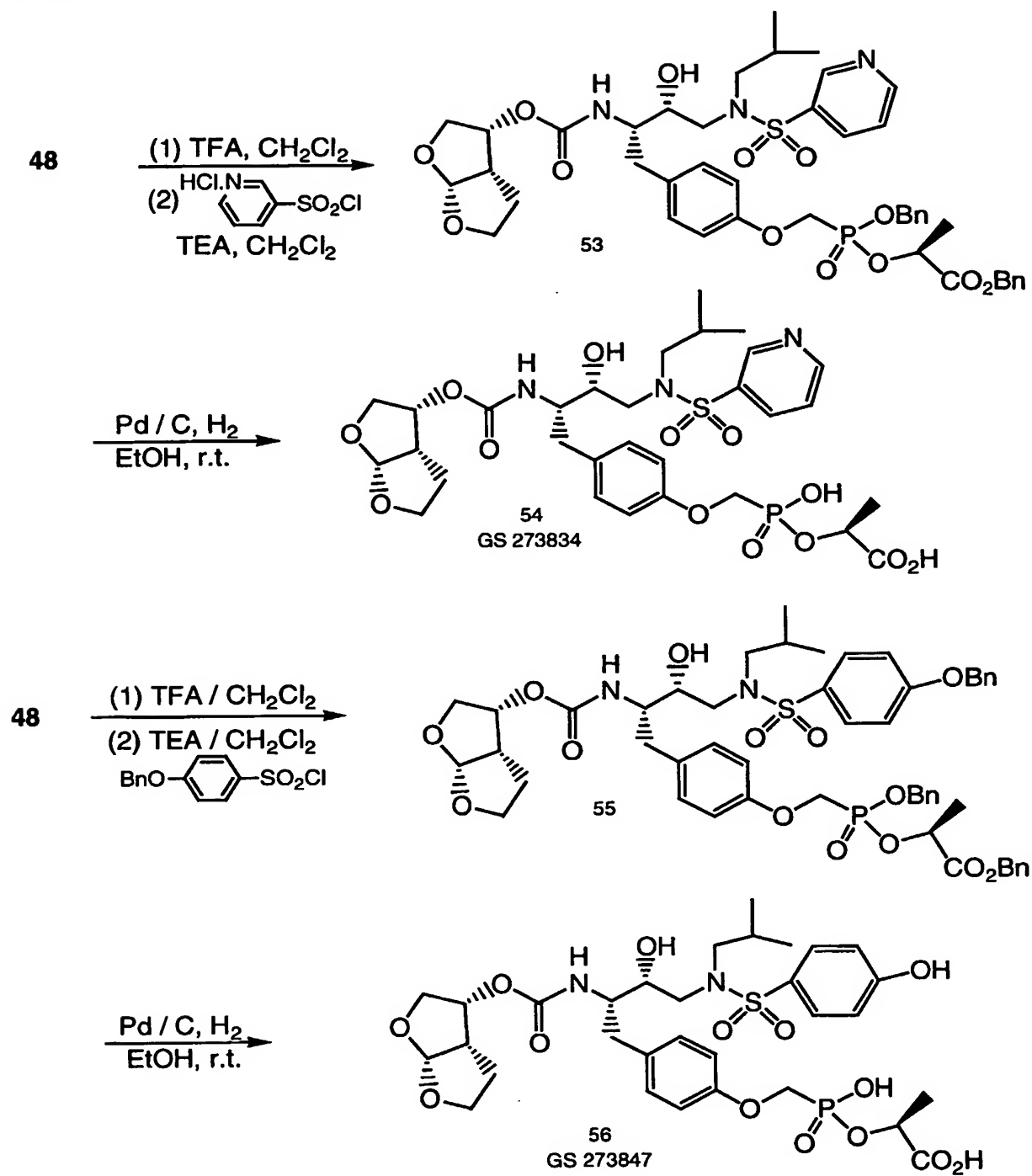
Scheme 12



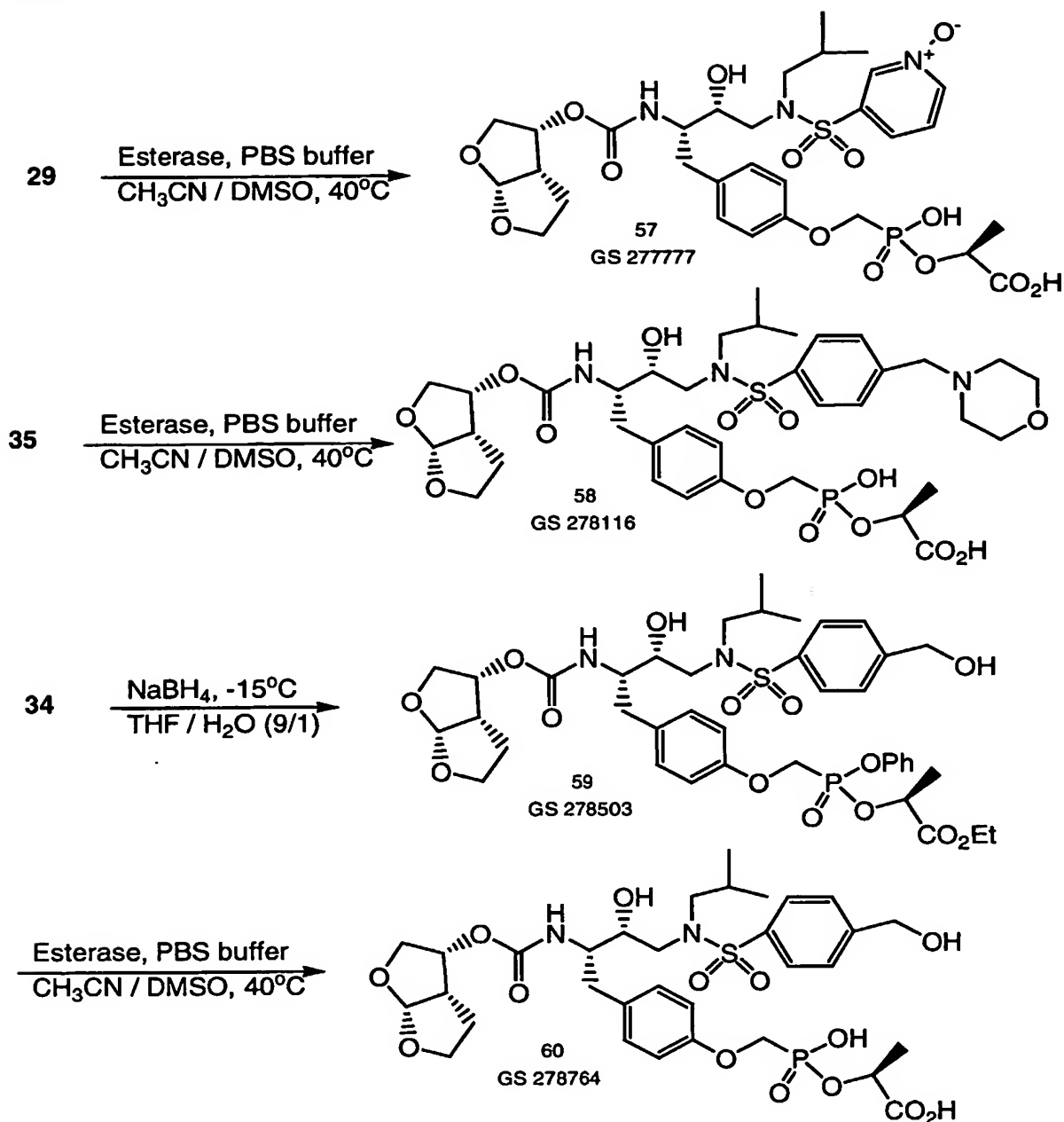
Scheme 13



Scheme 14



Scheme 15



Example 42

- 5 Monobenzylphosphonate 47: A solution of 6 (2.00 g, 2.55 mmol) and DABCO (0.29 g, 2.55 mmol) in toluene (10 mL) was heated to reflux for 2 h. The solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with H₂O, saturated NaCl, dried with Na₂SO₄, filtered, and concentrated.

The crude product was dried under vacuum to give the monobenzylphosphonate (1.68 g, 95%) as a white solid.

Example 43

5 Monophospholactate 48: To a solution of 47 (2.5 g, 3.61 mmol) and benzyl-(S)-(-)-lactate (0.87 mL, 5.42 mmol) in DMF (12 mL) was added PyBop (2.82 g, 5.42 mmol) and *N,N*-diisopropylethylamine (2.51 mL, 14.44 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated. The residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with H₂O, saturated NaCl, dried with Na₂SO₄, filtered,
10 and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the monophospholactate (1.58 g, 51%) as a white solid.

Example 44

Monophospholactate 49: A solution of 48 (0.30 g, 0.35 mmol) in CH₂Cl₂ (0.6 mL) at 0°C
15 was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH₂Cl₂ (2 mL) and cooled to 0°C. Triethylamine (0.20 mL, 1.40
20 mmol) was added followed by the treatment of benzenesulfonyl chloride (62 mg, 0.35 mmol). The solution was stirred at 0°C for 30 min and then warmed to room temperature for 30 min. The product was partitioned between CH₂Cl₂ and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the
25 monophospholactate (0.17 g, 53%) as a white solid.

Example 45

Metabolite X 50: To a solution of 49 (80 mg, 0.09 mmol) in EtOH (6 mL) and EtOAc (2 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H₂ atmosphere
30 (balloon) at room temperature for 8 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl₃ and dried under vacuum to give the metabolite X (61 mg, 95%, GS 224342) as a white solid: ¹H NMR (CD₃OD) δ 7.83 (d, J = 6.9 Hz, 2H), 7.65-7.58 (m, 3H), 7.18 (d, J = 7.8 Hz, 2H), 6.90 (d, J = 7.8 Hz, 2H), 5.59

(d, $J = 4.8$ Hz, 1H), 5.0 (m, 1H), 4.27 (d, $J = 10.2$ Hz, 2H), 3.95-3.68 (m, 6H), 3.45 (dd, 1H), 3.18-2.84 (m, 6H), 2.50 (m, 1H), 2.02 (m, 1H), 1.6-1.38 (m, 5H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.88 (d, $J = 6.3$ Hz, 3H); ^{31}P NMR (CD_3OD), δ 18.0.

5 Example 46

Monophospholactate 51: A solution of 48 (0.28 g, 0.33 mmol) in CH_2Cl_2 (0.6 mL) at 0°C was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C . Triethylamine (0.18 mL, 1.32 mmol) was added followed by the treatment of 4-fluorobenzenesulfonyl chloride (64 mg, 0.33 mmol). The solution was stirred at 0°C for 30 min and then warmed to room temperature for 30 min. The product was partitioned between CH_2Cl_2 and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.16 g, 52%) as a white solid.

Example 47

20 Metabolite X 52: To a solution of 51 (80 mg, 0.09 mmol) in EtOH (6 mL) and EtOAc (2 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H_2 atmosphere (balloon) at room temperature for 8 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl_3 and dried under vacuum to give the metabolite X (61 mg, 95%, GS 224343) as a white solid: ^1H NMR (CD_3OD) δ 7.9 (dd, 2H), 7.32 (m, 2H), 7.18 (dd, 2H), 6.90 (dd, 2H), 5.59 (d, $J = 5.4$ Hz, 1H), 5.0 (m, 1H), 4.28 (d, $J = 10.2$ Hz, 2H), 3.95-3.72 (m, 6H), 3.44 (dd, 1H), 3.15-2.85 (m, 6H), 2.5 (m, 1H), 2.02 (m, 1H), 1.55-1.38 (m, 5H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.88 (d, $J = 6.3$ Hz, 3H). ^{31}P NMR (CD_3OD) δ 18.2.

30 Example 48

Monophospholactate 53: A solution of 48 (0.20 g, 0.24 mmol) in CH_2Cl_2 (0.6 mL) at 0°C was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at 0°C and

then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C . Triethylamine (0.16 mL, 1.20 mmol) was added followed by the treatment of hydrogen chloride salt of 3-pyridinysulfonyl chloride (50 mg, 0.24 mmol). The solution was stirred at 0°C for 30 min and then warmed to room temperature for 30 min. The product was partitioned between CH_2Cl_2 and H_2O . The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.11 g, 53%) as a white solid.

Example 49

Metabolite X 54: To a solution of 53 (70 mg, 0.09 mmol) in EtOH (5 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H_2 atmosphere (balloon) at room temperature for 5 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl_3 and dried under vacuum to give the metabolite X (53 mg, 95%, GS 273834) as a white solid: ^1H NMR (CD_3OD) δ 8.99 (s, 1H), 8.79 (d, J = 4.2 Hz, 1H), 8.29 (d, J = 7.5 Hz, 1H), 7.7 (m, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 7.8 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.0 (m, 1H), 4.28 (d, J = 9.9 Hz, 2H), 3.97-3.70 (m, 6H), 3.44 (dd, 1H), 3.17-2.85 (m, 6H), 2.5 (m, 1H), 2.03 (m, 1H), 1.65-1.38 (m, 5H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H). ^{31}P NMR (CD_3OD) δ 17.8.

Example 50

Monophospholactate 55: A solution of 48 (0.15 g, 0.18 mmol) in CH_2Cl_2 (1 mL) at 0°C was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C . Triethylamine (0.12 mL, 0.88 mmol) was added followed by the treatment of 4-benzyloxybenzenesulfonyl chloride (50 mg, 0.18 mmol). The solution was stirred at 0°C for 30 min and then warmed to room temperature for 30 min. The product was partitioned between CH_2Cl_2 and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and

concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the monophospholactate (0.11 g, 63%) as a white solid.

Example 51

- 5 Metabolite X 56: To a solution of 55 (70 mg, 0.07 mmol) in EtOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl₃ and dried under vacuum to give the metabolite X (46 mg, 90%, GS 273847) as a white solid: ¹H NMR (CD₃OD), δ 7.91 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 6.91 (m, 4H), 5.59 (d, J = 5.1 Hz, 1H), 5.0 (m, 1H), 4.27 (d, J = 10.2 Hz, 2H), 3.97-3.74 (m, 6H), 3.4 (dd, 1H), 3.17-2.8 (m, 6H), 2.5 (m, 1H), 2.0 (m, 1H), 1.6-1.38 (m, 5H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); ³¹P NMR (CD₃OD) δ 17.9.

15 Example 52

- Metabolite X 57: To a suspension of 29 (40 mg, 0.05 mmol) in CH₃CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (200 μL). The suspension was heated to 40°C for 48 h. The reaction mixture was concentrated, suspended in MeOH and filtered. The filtrate was concentrated and purified by HPLC to give the metabolite X (20 mg, 57%, GS 277777) as a white solid: ¹H NMR (CD₃OD) δ 8.68 (s, 1H), 8.47 (d, J = 6.0 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.68 (m, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 8.4 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.0 (m, 1H), 4.23 (d, J = 10.5 Hz, 2H), 3.97-3.68 (m, 6H), 3.45 (dd, 1H), 3.15-2.87 (m, 6H), 2.46 (m, 1H), 2.0 (m, 1H), 1.6-1.38 (m, 5H), 0.95 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H); ³¹P NMR (CD₃OD) δ 17.2.

25

Example 53

- Metabolite X 58: To a suspension of 35 (60 mg, 0.07 mmol) in CH₃CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (400 μL). The suspension was heated to 40°C for 3 days. The reaction mixture was concentrated, suspended in MeOH and filtered.
- 30 The filtrate was concentrated and purified by HPLC to give the metabolite X (20 mg, 38%, GS 278116) as a white solid: ¹H NMR (CD₃OD) δ 7.74 (d, J = 6.9 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.1 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.0 (m,

2H), 4.41 (m, 2H), 4.22 (m, 2H), 3.97-3.65 (m, 12H), 3.15-2.9 (m, 8H), 2.75 (m, 1H), 2.0 (m, 1H), 1.8 (m, 2H), 1.53 (d, J = 6.9 Hz, 3H), 0.88 (m, 6H).

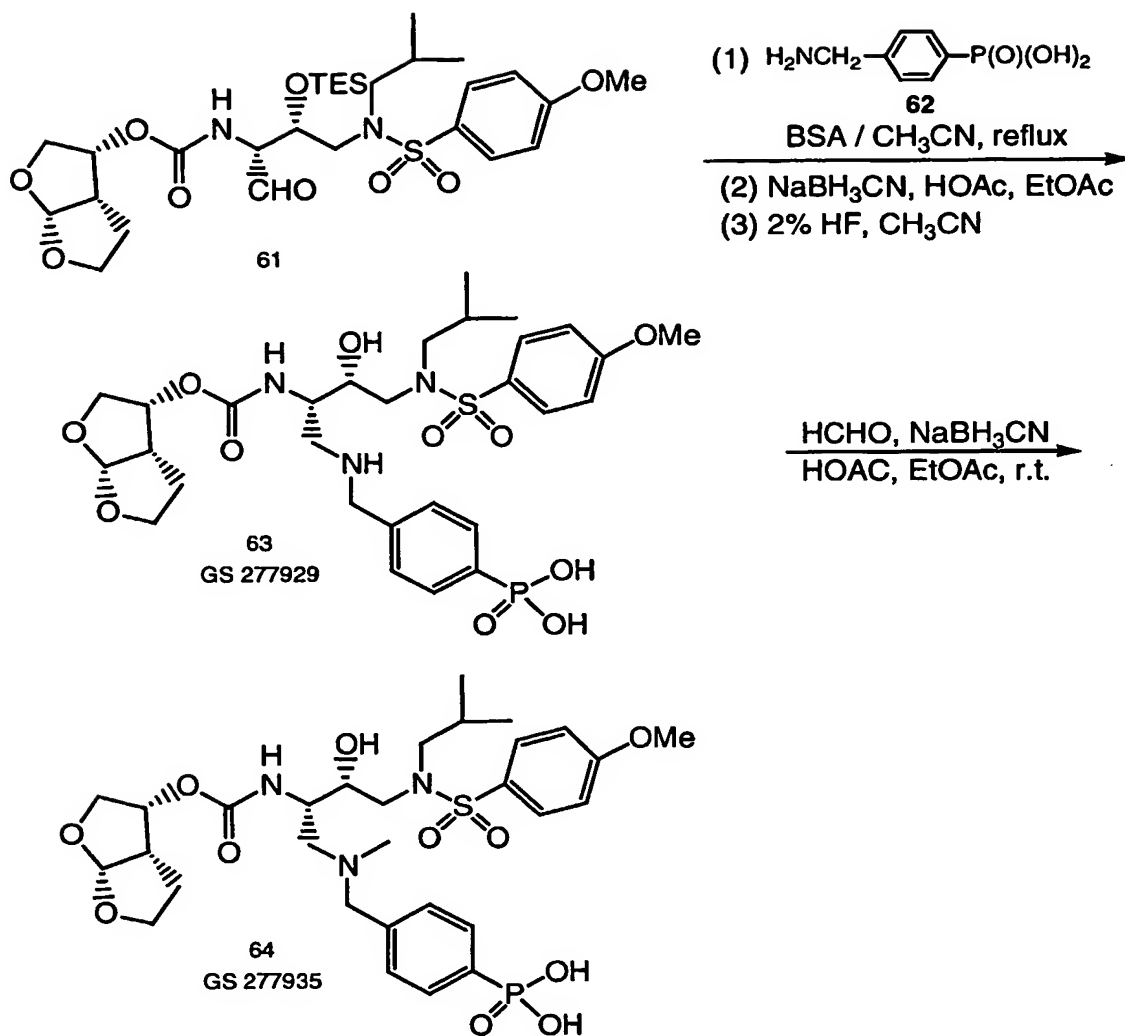
Example 54

- 5 Monophospholactate 59: A solution of 34 (2.10 g, 2.48 mmol) in THF (72 mL) and H₂O (8 mL) at -15°C was treated with NaBH₄ (0.24 g, 6.20 mmol). The reaction mixture was stirred for 10 min at -15°C. The reaction was quenched with 5% aqueous NaHSO₃ and extracted with CH₂Cl₂ (3 x). The combined organic layers were washed with H₂O, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on
- 10 silica gel (5% 2-propanol/CH₂Cl₂) to give monophospholactate (1.89 g, 90%, GS 278053, 1:1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 7.64 (m, 2H), 7.51(m, 2H), 7.38-7.19 (m, 7H), 6.92 (m, 2H), 5.69 (d, J = 4.8 Hz, 1H), 5.15 (m, 2H), 4.76 (s, 2H), 4.54 (d, J = 10.5 Hz, 1H), 4.44 (m, 1H), 4.2 (m, 2H), 4.04-3.68 (m, 6H), 3.06-2.62 (m, 7H), 1.8 (m, 3H), 1.62-1.5 (dd, 3H), 1.25 (m, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.3 Hz, 3H); ³¹P
- 15 NMR (CDCl₃) δ 17.4, 15.4.

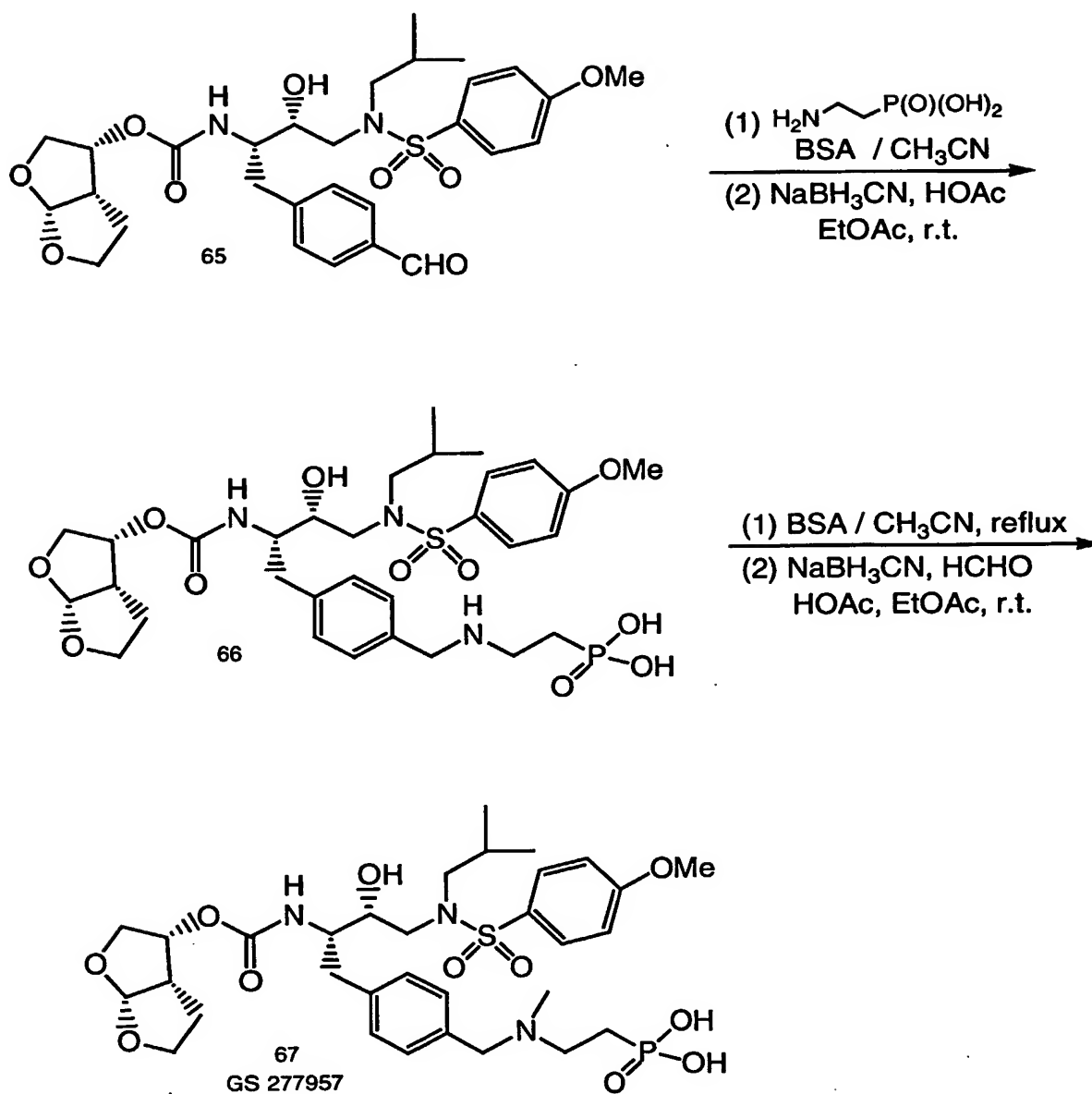
Example 55

- Metabolite X 60: To a suspension of 59 (70 mg, 0.08 mmol) in CH₃CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (600 μL). The suspension was heated
- 20 to 40°C for 36 h. The reaction mixture was concentrated, suspended in MeOH and filtered. The filtrate was concentrated and purified by HPLC to give the metabolite X (22 mg, 36%, GS 278764) as a white solid: ¹H NMR (CD₃OD) δ 7.78 (dd, 2H), 7.54 (dd, 2H), 7.15 (m, 2H), 6.9 (m, 2H), 5.57 (d, 1H), 5.0 (m, 2H), 4.65 (m, 4H), 4.2 (m, 2H), 3.9-3.53 (m, 6H), 3.06-2.82 (m, 6H), 2.5 (m, 1H), 2.0 (m, 2H), 1.62-1.35 (m, 3H), 0.94 (m, 6H).

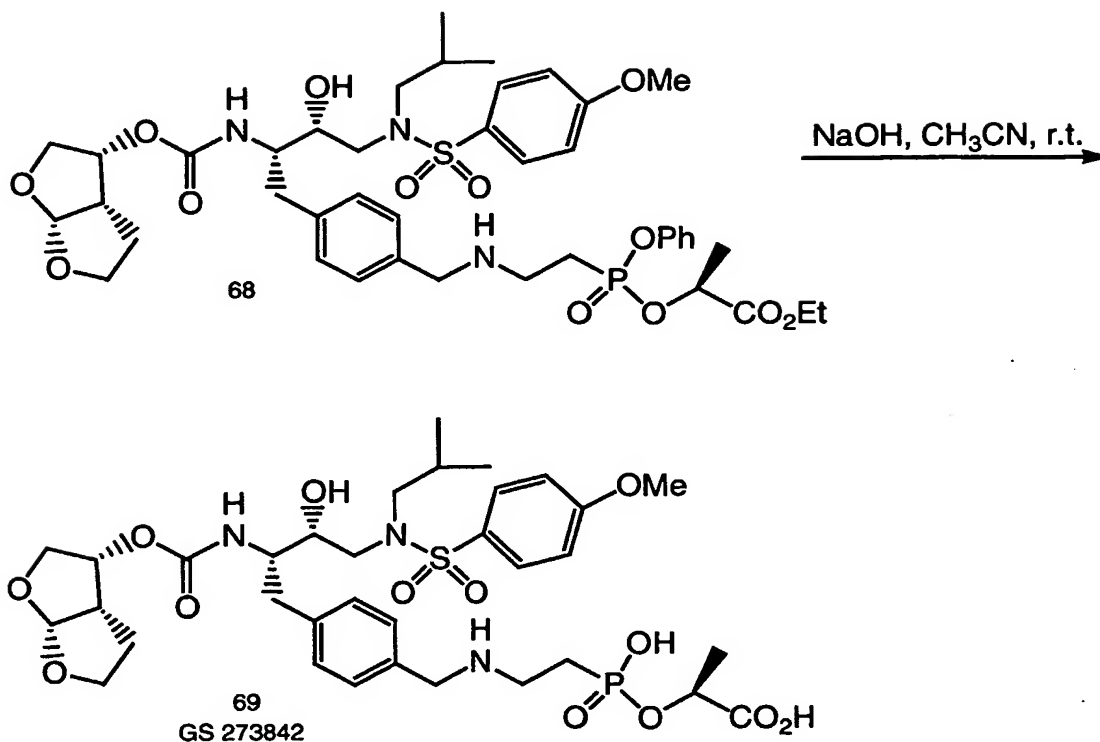
Scheme 16



Scheme 17



Scheme 18

Example 56

- 5 Phosphonic Acid 63: Compound 62 (0.30 g, 1.12 mmol) was dissolved in CH₃CN (5 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 2.2 mL, 8.96 mmol) was added. The reaction mixture was heated to reflux for 2 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (4 mL) and cooled to 0°C. Aldehyde 61 (0.20 g, 0.33
- 10 mmol), AcOH (0.18 mL, 3.30 mmol), and NaBH₃CN (0.20 g, 3.30 mmol) were added. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched with H₂O, stirred for 30 min, filtered, and concentrated. The crude product was dissolved in CH₃CN (13 mL) and 48% aqueous HF (0.5 mL) was added. The reaction mixture was stirred at room temperature for 2 h and concentrated. The crude product was
- 15 purified by HPLC to give the phosphonic acid (70 mg, 32%, GS 277929) as a white solid: ¹H NMR (CD₃OD) δ 7.92 (dd, 2H), 7.73 (d, J = 8.7 Hz, 2H), 7.63 (dd, 2H), 7.12 (d, J = 8.7 Hz, 2H), 5.68 (d, J = 5.1 Hz, 1H), 5.13 (m, 1H), 4.4 (m, 2H), 4.05-3.89 (m, 8H), 3.75 (m, 1H), 3.5 (m, 1H), 3.37 (m, 1H), 3.23-3.0 (m, 3H), 2.88-2.7 (m, 2H), 2.2 (m, 1H), 1.8 (m, 2H), 0.92 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); ³¹P NMR (CD₃OD) δ 14.5.

Example 57

Phosphonic Acid 64: A solution of 63 (50 mg, 0.07 mmol) and formaldehyde (60 mg, 0.70 mmol) in EtOAc (2 mL) was treated with HOAc (43 μ L, 0.70 mmol) and NaBH₃CN (47 mg, 0.7 mmol). The reaction mixture was stirred at room temperature for 26 h. The reaction was quenched with H₂O, stirred for 20 min, and concentrated. The crude product was purified by HPLC to give the phosphonic acid (15 mg, 29%, **GS 277935**) as a white solid: ¹H NMR (CD₃OD) δ 7.93 (m, 2H), 7.75 (m, 2H), 7.62 (m, 2H), 7.11 (m, 2H), 5.66 (m, 1H), 5.13 (m, 1H), 4.4 (m, 2H), 4.05-3.89 (m, 8H), 3.75 (m, 2H), 3.09-2.71 (m, 6H), 2.2 (m, 1H), 1.9 (m, 5H), 0.92 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); ³¹P NMR (CD₃OD) δ 14.0.

Example 58

Phosphonic Acid 66: 2-Aminoethylphosphonic acid (2.60 g, 21.66 mmol) was dissolved in CH₃CN (40 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 40 mL) was added. The reaction mixture was heated to reflux for 2 h and cooled to room temperature and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (40 mL). Aldehyde 65 (1.33 g, 2.25 mmol), AcOH (1.30 mL, 22.5 mmol) and NaBH₃CN (1.42 g, 22.5 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H₂O, stirred for 1 h, filtered, and concentrated. The residue was dissolved in MeOH and filtered. The crude product was purified by HPLC to give the phosphonic acid (1.00 g, 63%) as a white solid.

Example 59

Phosphonic Acid 67: Phosphonic acid 66 (0.13 g, 0.19 mmol) was dissolved in CH₃CN (4 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.45 mL, 1.90 mmol) was added. The reaction mixture was heated to reflux for 2 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (3 mL). Formaldehyde (0.15 mL, 1.90 mmol), AcOH (0.11 mL, 1.90 mmol) and NaBH₃CN (63 mg, 1.90 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H₂O, stirred for 6 h, filtered, and concentrated. The residue was dissolved in MeOH and filtered. The crude product was purified by HPLC to give the phosphonic acid (40 mg, 30%, **GS**

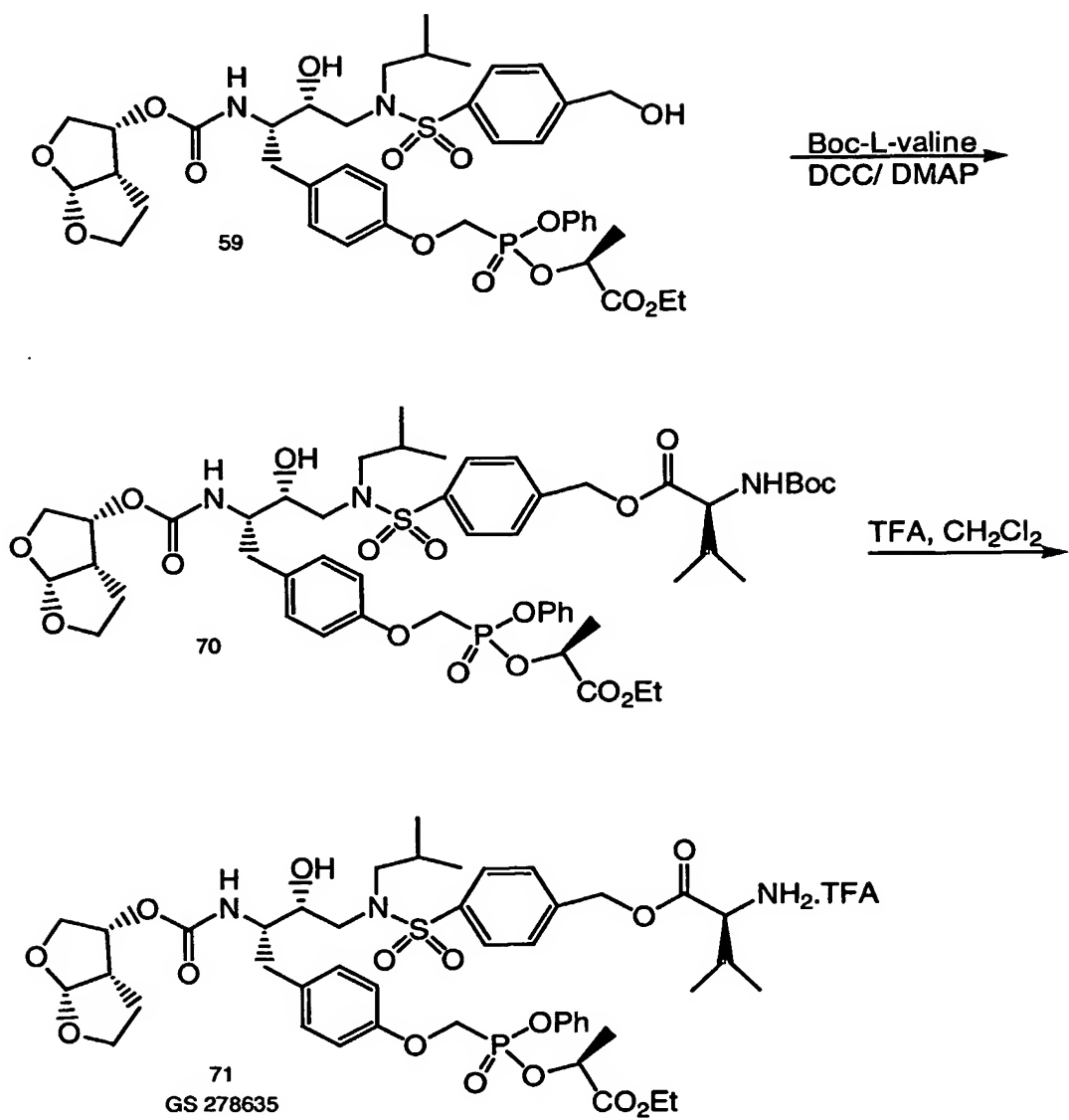
277957) as a white solid: ^1H NMR (CD_3OD) δ 7.78 (d, $J = 8.4$ Hz, 2H), 7.4 (m, 4H), 7.09 (d, $J = 8.4$ Hz, 2H), 5.6 (d, $J = 5.1$ Hz, 1H), 4.33 (m, 2H), 3.95-3.65 (m, 9H), 3.5-3.05 (m, 6H), 2.91-2.6 (m, 7H), 2.0 (m, 3H), 1.5 (m, 2H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.87 (d, $J = 6.3$ Hz, 3H); ^{31}P NMR (CD_3OD) δ 19.7.

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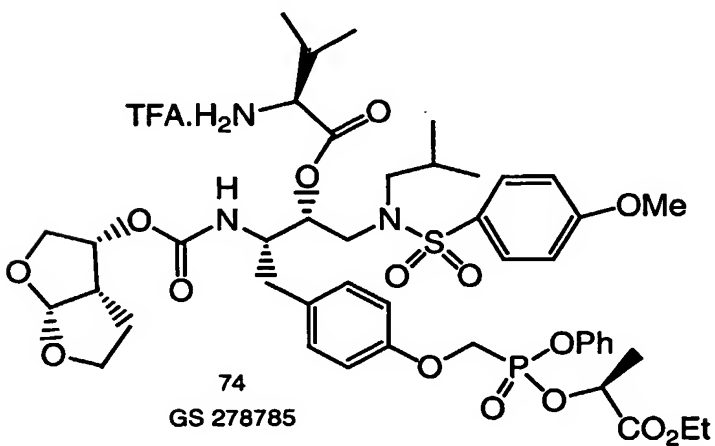
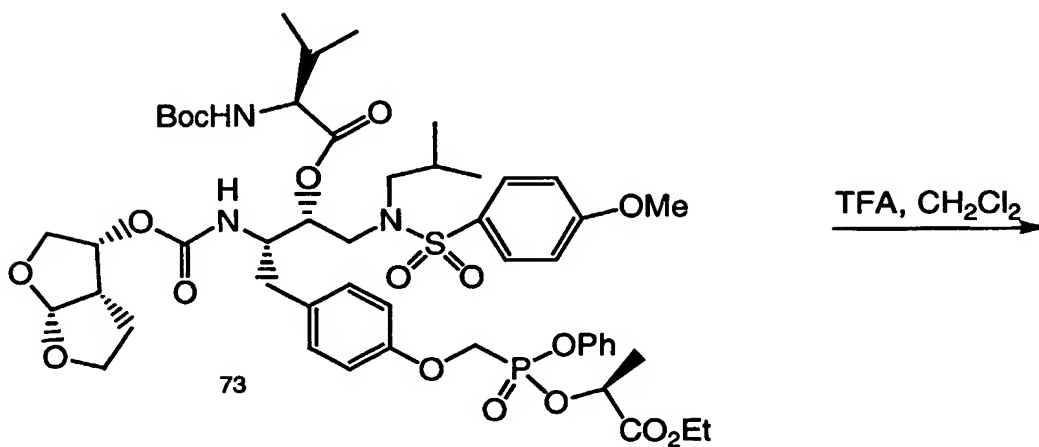
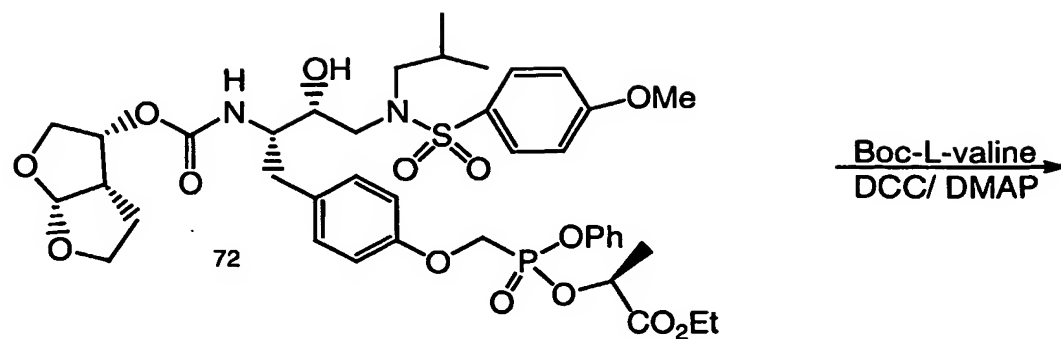
Example 60

Metabolite X 69: Monophospholactate 68 (1.4 g, 1.60 mmol) was dissolved in CH_3CN (20 mL) and H_2O (20 mL). 1.0 N NaOH (3.20 mL, 3.20 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h and cooled to 0°C . The reaction mixture was acidified to pH = 1-2 with 2 N HCl (1.6 mL, 3.20 mmol). The solvent was evaporated under reduced pressure. The crude product was purified by HPLC to give the metabolite X (0.60 g, 49%, GS 273842) as a white solid: ^1H NMR ($\text{DMSO}-d_6$) δ 7.72 (d, $J = 8.7$ Hz, 2H), 7.33 (m, 4H), 7.09 (d, $J = 9.0$ Hz, 2H), 5.52 (d, $J = 5.7$ Hz, 1H), 5.1 (broad, s, 1H), 4.85 (m, 1H), 4.63 (m, 1H), 4.13 (m, 2H), 3.8 (m, 5H), 3.6 (m, 4H), 3.36 (m, 1H), 3.03 (m, 4H), 2.79 (m, 3H), 2.5 (m, 1H), 2.0 (m, 3H), 1.5-1.3 (m, 5H), 0.85 (d, $J = 6.6$ Hz, 3H), 0.79 (d, $J = 6.6$ Hz, 3H); ^{31}P NMR ($\text{DMSO}-d_6$) δ 21.9.

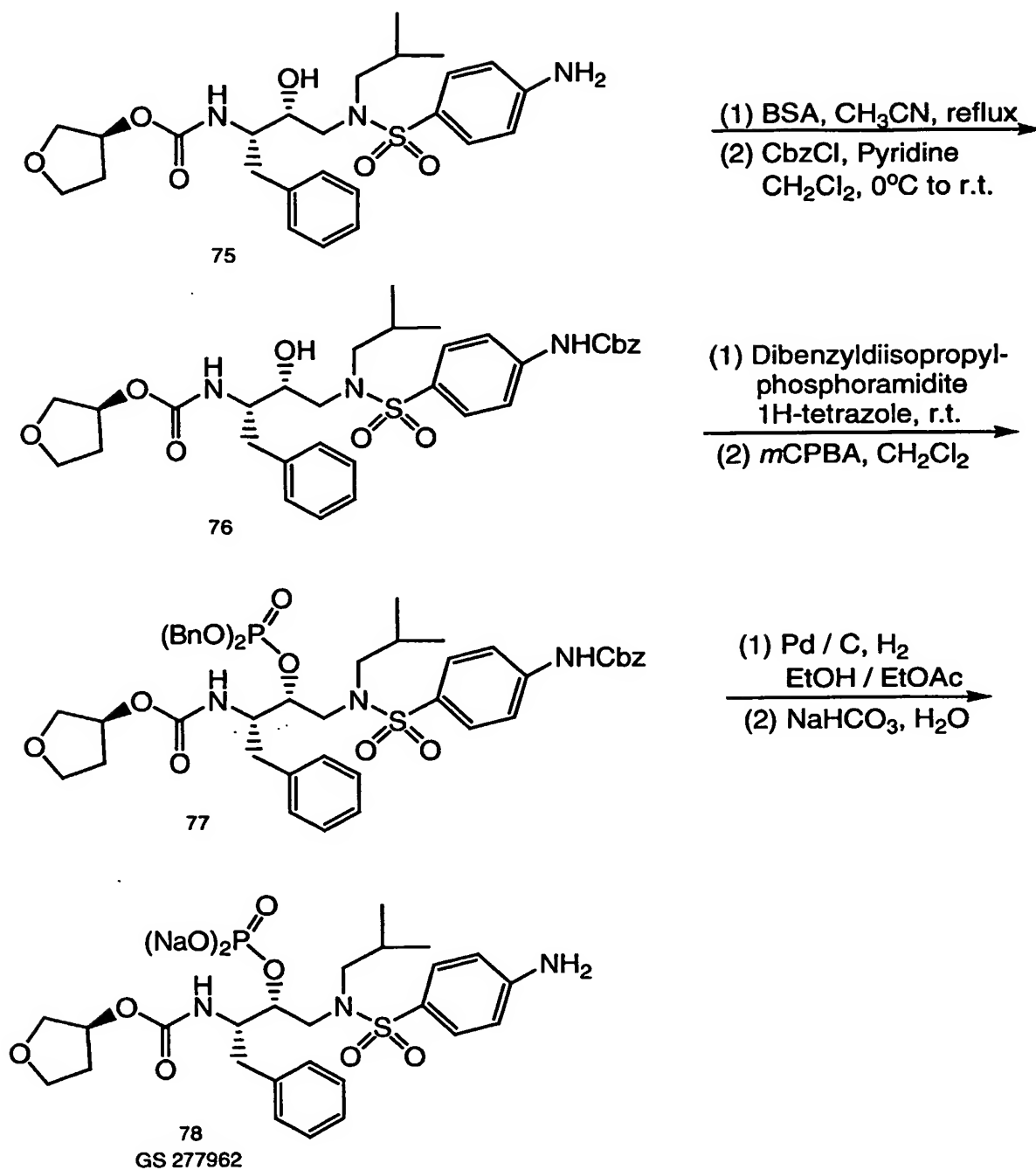
Scheme 19



Scheme 20



Scheme 21

5 Example 61

Monophospholactate 70: A solution of 59 (1.48 g, 1.74 mmol) and Boc-L-valine (0.38 g, 1.74 mmol) in CH₂Cl₂ (30 mL) at 0°C was treated with 1,3- dicyclohexylcarbodiimide (0.45 g, 2.18 mmol) and 4-dimethylaminopyridine (26 mg, 0.21 mmol). The reaction mixture was stirred at 0°C for 1 h and then warmed to room temperature for 2 h. The product was

partitioned between CH_2Cl_2 and 0.2 N HCl. The organic layer was washed with H_2O , dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ CH_2Cl_2) to give the monophospholactate (1.65 g, 90%) as a white solid.

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Example 62

Monophospholactate 71: A solution of 70 (1.65 g, 1.57 mmol) in CH_2Cl_2 (8 mL) at 0°C was treated with trifluoroacetic acid (4 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ CH_2Cl_2) to give the monophospholactate (1.42 g, 85%, GS 278635, 2/3 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 7.73 (m, 2H), 7.49 (d, $J = 7.2$ Hz, 2H), 7.4-7.1 (m, 7H), 6.89 (m, 2H), 5.64 (m, 1H), 5.47 (m, 1H), 5.33-5.06 (m, 4H), 4.57-4.41 (m, 2H), 4.2 (m, 2H), 3.96-3.7 (m, 7H), 3.15-2.73 (m, 7H), 2.38 (m, 1H), 1.9 (m, 1H), 1.7 (m, 1H), 1.63-1.5 (m, 4H), 1.24 (m, 3H), 1.19 (m, 6H), 0.91 (d, 3H), 0.88 (d, 3H); ^{31}P NMR (CDCl_3) δ 17.3, 15.4.

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Example 63

Monophospholactate 73: A solution of 72 (0.43 g, 0.50 mmol) and Boc-L-valine (0.11 g, 0.50 mmol) in CH_2Cl_2 (6 mL) was treated with 1,3-dicyclohexylcarbodiimide (0.13 g, 0.63 mmol) and 4-dimethylaminopyridine (62 mg, 0.5 mmol). The reaction mixture was stirred at room temperature overnight. The product was partitioned between CH_2Cl_2 and 0.2 N HCl. The organic layer was washed with H_2O , dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.45 g, 85%) as a white solid.

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Example 64

Monophospholactate 74: A solution of 73 (0.44 g, 0.42 mmol) in CH_2Cl_2 (1 mL) at 0°C was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ CH_2Cl_2) to give the monophospholactate (0.40 g, 90%, GS 278785, 1:1 diastereomeric mixture) as a white solid:

30

¹H NMR (CDCl₃) δ 7.69 (d, J = 8.4 Hz, 2H), 7.34-7.2 (m, 7H), 6.98 (d, J = 8.4 Hz, 2H), 6.88 (m, 2H), 6.16 (m, 1H), 5.64 (m, 1H), 5.46 (m, 1H), 5.2-5.0 (m, 2H), 4.5 (m, 2H), 4.2 (m, 3H), 4.0-3.4 (m, 9H), 3.3 (m, 1H), 3.0-2.8 (m, 5H), 2.5 (m, 1H), 1.83 (m, 1H), 1.6-1.5 (m, 5H), 1.25 (m, 3H), 1.15 (m, 6H), 0.82 (d, J = 6.0 Hz, 3H), 0.76 (d, J = 6.0 Hz, 3H); ³¹P NMR (CDCl₃) δ 17.3, 15.5.

Example 65

Cbz Amide 76: Compound 75 (0.35 g, 0.69 mmol) was dissolved in CH₃CN (6 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.67 mL, 2.76 mmol) was added. The reaction mixture was heated to reflux for 1 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in CH₂Cl₂ (3 mL) and cooled to 0°C. Pyridine (0.17 mL, 2.07 mmol) and benzyl chloroformate (0.12 mL, 0.83 mmol) were added. The reaction mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction was quenched with MeOH (5 mL) and 10% HCl (20 mL) at 0°C and stirred for 1 h. The product was extracted with CH₂Cl₂, washed with brine, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the CBz amide (0.40 g, 90%) as a white solid.

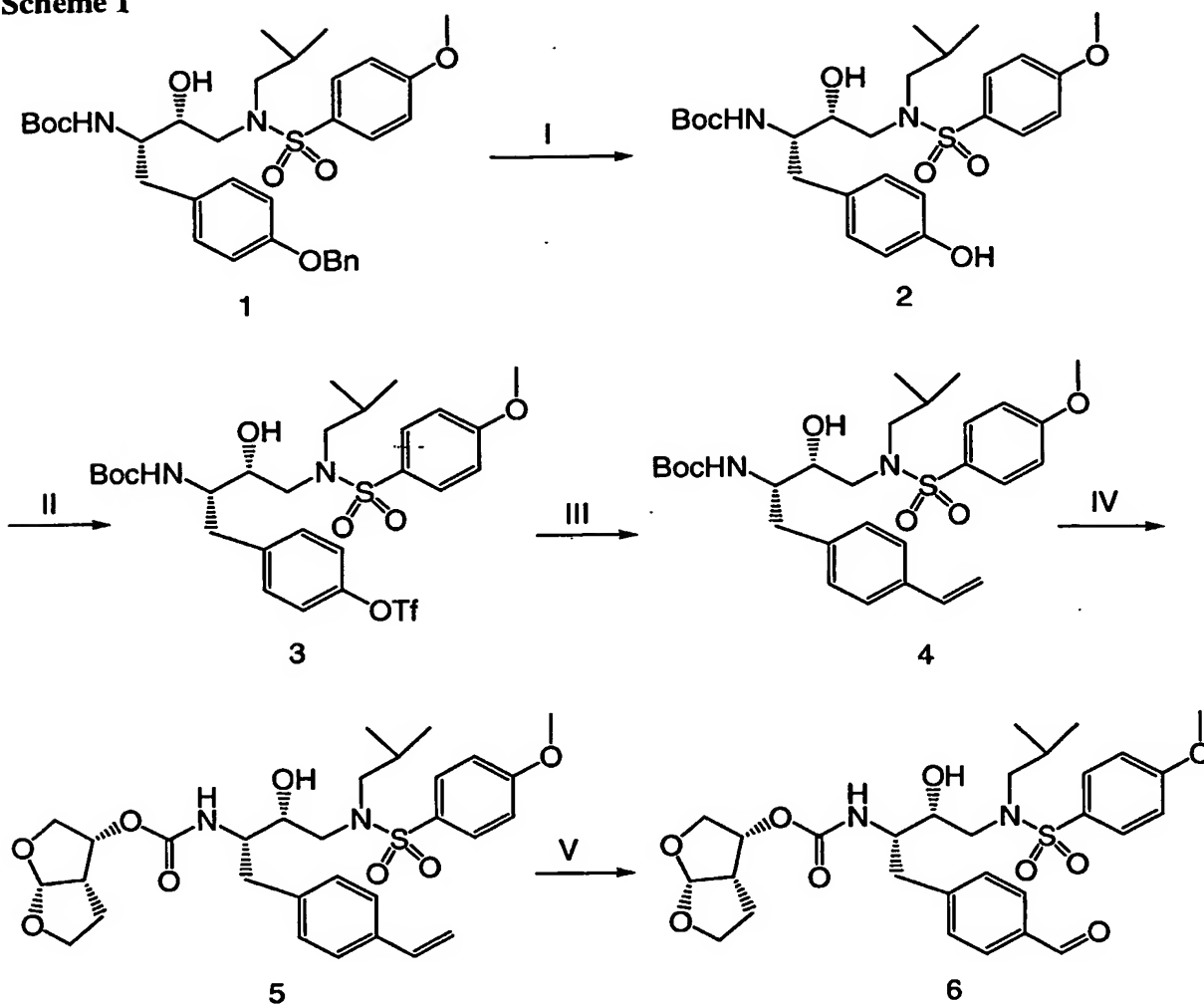
Example 66

Dibenzylphosphonate 77: A solution of 76 (0.39 g, 0.61 mmol) and 1*H*-tetrazole (54 mg, 0.92 mmol) in CH₂Cl₂ (8 mL) was treated with dibenzyl diisopropylphosphoramidite (0.32 g, 0.92 mmol) and stirred at room temperature overnight. The solution was cooled to 0°C, treated with *m*CPBA, stirred for 1 h at 0°C and then warmed to room temperature for 1 h. The reaction mixture was poured into a mixture of aqueous Na₂SO₃ and NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed with H₂O, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the dibenzylphosphonate (0.42 g, 76%) as a white solid.

Example 67

Disodium Salt of Phosphonic Acid 78: To a solution of 77 (0.18 g, 0.20 mmol) in EtOH (20 mL) and EtOAc (4 mL) was added 10% Pd/C (40 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through

a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (0.11 g, 95%) which was dissolved in H₂O (4 mL) and treated with NaHCO₃ (32 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (0.12 g, 99%, GS 277962) as a white solid: ¹H NMR (D₂O) δ 7.55 (dd, 2H), 7.2 (m, 5H), 7.77 (dd, 2H), 4.65 (m, 1H), 4.24 (m, 1H), 4.07 (m, 1H), 3.78-2.6 (m, 12H), 1.88-1.6 (m, 3H), 0.75 (m, 6H).

Scheme 1

I. $\text{H}_2/10\%\text{Pd-C}/\text{EtOAc-EtOH}$; II. $\text{Tf}_2\text{NPh}/\text{Cs}_2\text{CO}_3$;
 III. $\text{Bu}_3\text{SnCH=CH}_2/\text{PdCl}_2(\text{PPh}_3)_2/\text{LiCl}/\text{DMF}/90^\circ\text{C}$;
 IV. a. $\text{TFA}/\text{CH}_2\text{Cl}_2$; b. Bisfurancarboxylic acid/ $i\text{-Pr}_2\text{NEt}/\text{DMAP}$;
 V. $\text{NaIO}_4/\text{OsO}_4/\text{EtOAc-H}_2\text{O}$

Example 1

- 5 Compound 1 was prepared by methods from Examples herein.

Example 2

Compound 2: To a solution of compound 1 (47.3 g) in EtOH/EtOAc (1000 mL/500 mL) was added 10% Pd-C (5 g). The mixture was hydrogenated for 19 hours. Celite was added and

the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and was washed with ethyl acetate. Concentration gave compound 2 (42.1 g).

Example 3

5 Compound 3: To a solution of compound 2 (42.3 g, 81 mmol) in CH_2Cl_2 (833 mL) was added N-phenyltrifluoromethanesulfonimide (31.8 g, 89 mmol), followed by cesium carbonate (28.9 g, 89 mmol). The mixture was stirred for 24 hours. The solvent was removed under reduced pressure, and ethyl acetate was added. The reaction mixture was washed with water (3x) and brine (1x), and was dried over MgSO_4 . Purification by flash
10 column chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc} = 13/1$) gave compound 3 (49.5 g) as a white powder.

Example 4

Compound 4: To a solution of compound 3 (25.2, 38.5 mmol) in DMF (240 mL) was added
15 lithium chloride (11.45 g, 270 mmol), followed by dichlorobis(triphenylphosphine) palladium(II) (540 mg, 0.77 mmol). The mixture was stirred for 3 minutes under high vacuum and recharged with nitrogen. To the above solution was added tributylvinyltin (11.25 mL). The reaction mixture was heated at 90°C for 6 hours and cooled to 25°C . Water was added to the reaction, and the mixture was extracted with ethyl acetate (3X). The
20 combined organic layer was washed with water (6x) and brine, and dried over MgSO_4 . Concentration gave an oil. The oil was diluted with dichloromethane (40 mL), water (0.693 mL, 38.5 mmol) and DBU (5.76 mL, 38.5 mmol) were added. The mixture was stirred for 5 minutes, and subjected to flash column chromatography ($\text{hexanes}/\text{EtOAc} = 2.5/1$). Compound 4 was obtained as white solid (18.4 g).

25

Example 5

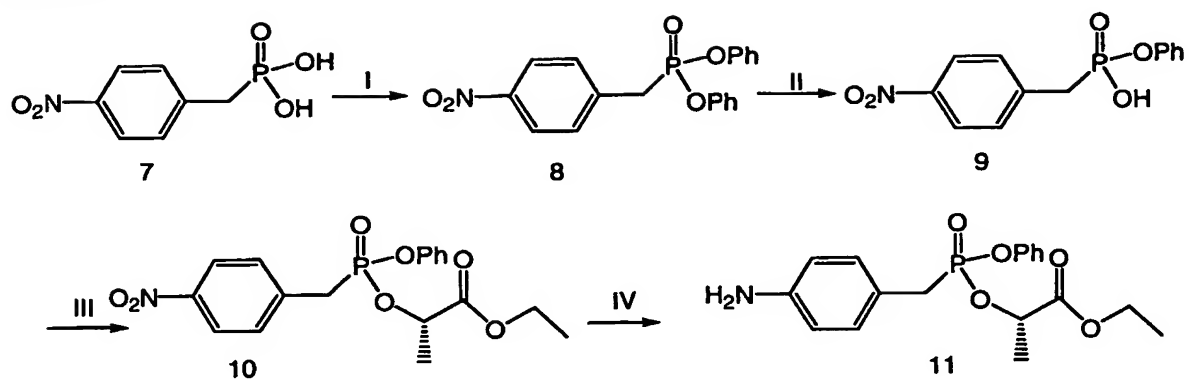
Compound 5: To a solution of compound 4 (18.4 g, 34.5 mmol) in CH_2Cl_2 (70 mL) at 0°C was added trifluoroacetic acid (35 mL). The mixture was stirred at 0°C for 2 hrs, and solvents were evaporated under reduced pressure. The reaction mixture was quenched with
30 saturated sodium carbonate solution, and was extracted with ethyl acetate (3x). The combined organic layer was washed with saturated sodium carbonate solution (1x), water (2x), and brine (1x), and dried over MgSO_4 . Concentration gave a solid. To a solution of the above solid in acetonitrile (220 mL) at 0°C was added bisfurancarboxylate (10.09 g, 34.2

mmol), followed by di-isopropylethylamine (12.0 mL, 69.1 mmol) and DMAP (843 mg, 6.9 mmol). The mixture was warmed to 25°C and stirred for 12 hours. Solvents were removed under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2X), 5% hydrochloric acid (2x), water (2x), 1N sodium hydroxide (2x), water (2x),
5 and brine (1x), and dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 1/1)) gave compound 5 (13.5 g).

Example 6

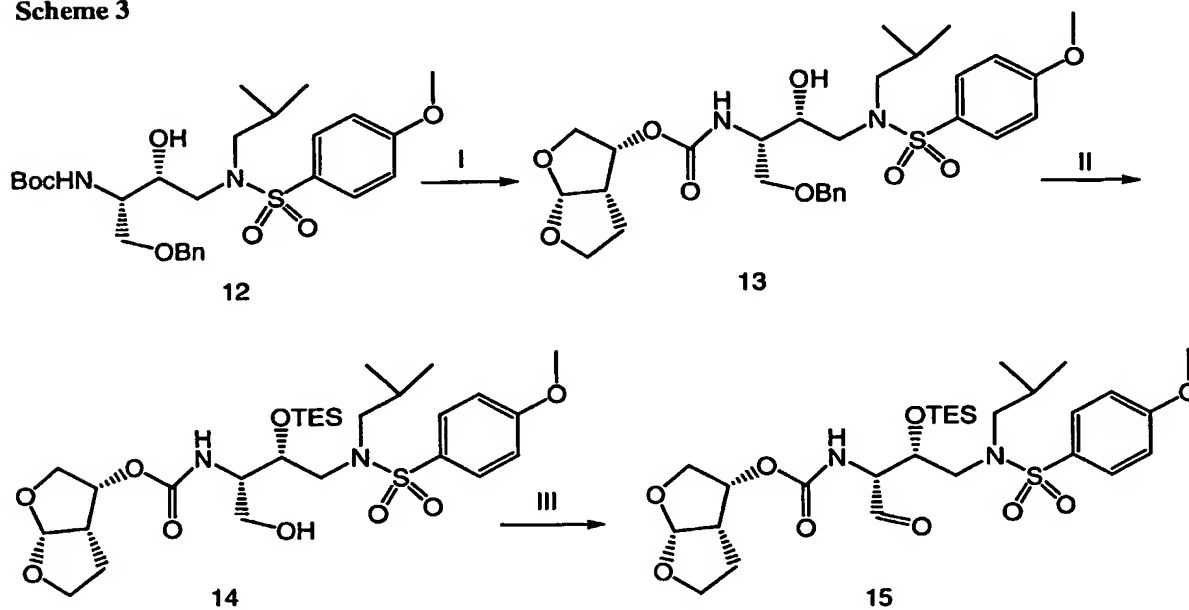
Compound 6: To a solution of compound 5 (13.5 g, 23 mmol) in ethyl acetate (135 mL) was
10 added water (135 mL), followed by 2.5% osmium tetroxide/tert-butanol (17 mL). Sodium periodate (11.5 g) was added in portions over 2 minutes period. The mixture was stirred for 90 minutes, and was diluted with ethyl acetate. The organic layer was separated and washed with water (3x) and brine (1x), and dried over MgSO₄. Purification by flash column
15 chromatography (hexanes/EtOAc = 1/2) gave compound 6 as white powder (12 g): ¹H NMR (CDCl₃) δ 9.98 (1 H, s), 7.82 (2 H, m), 7.75 (2 H, m), 7.43 (2 H, m), 6.99 (2 H, m), 5.64 (1 H, m), 5.02 (2 H, m), 4.0-3.8 (9 H, m), 3.2-2.7 (7 H, m), 1.9-1.4 (3 H, m), 0.94 (6 H, m).

Scheme 2

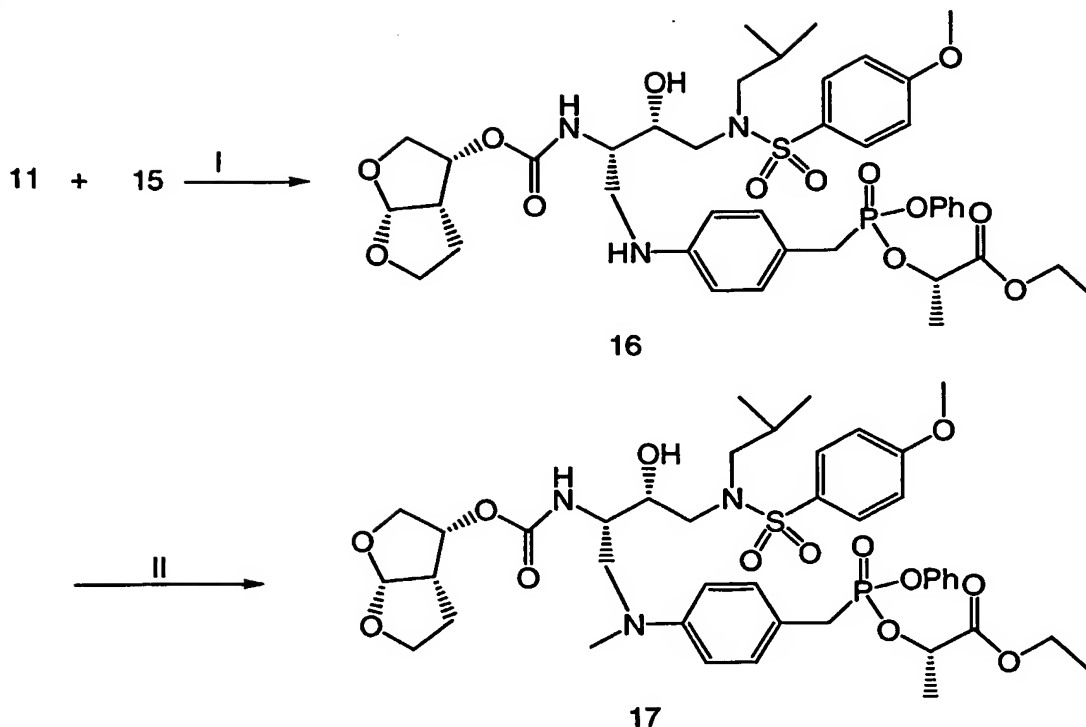


I. a. SOCl_2 /toluene/60 C; b. PhOH/pyridine; II. a. NaOH/THF/ H_2O ; b. HCl;
 III. b. SOCl_2 /toluene/60 C; c. ethyl lactate/pyridine; IV. H_2 /10% Pd-C/EtOAc

Scheme 3



I. a. TFA/ CH_2Cl_2 ; b. bisfurancarboxylate/ $i\text{-Pr}_2\text{NE}$ /DMAP; II. a. Et_3SiCl /imidazole/DMF;
 b. H_2 /20% Pd(OH) $_2$ -C/ $i\text{PrOH}$; III. Des-Martin reagent/ CH_2Cl_2

Scheme 4

I. a. $\text{NaBH}_3\text{CN}/\text{HOAc}/\text{EtOAc}$; b. 2% $\text{HF}/\text{CH}_3\text{CN}$;
 II. $\text{HCHO}/\text{NaBH}_3\text{CN}/\text{HOAc}/\text{EtOAc}$

Example 8

Compound 8: To the suspension of compound 7 (15.8 g, 72.5 mmol) in toluene (140 mL) was added DMF (1.9 mL), followed by thionyl chloride (53 mL, 725 mmol). The reaction mixture was heated at 60°C for 5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x), EtOAc, and CH_2Cl_2 (2x) to afford a brown solid. To the solution of the brown solid in CH_2Cl_2 at 0°C was added phenol (27.2 g, 290 mmol), followed by slow addition of pyridine (35 mL, 435 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO_4 . Concentration gave a dark oil, which was purified by flash column chromatography (hexanes/EtOAc = 4/1 to 1/1) to afford compound 8 (12.5 g).

Example 9

Compound 9: To a solution of compound 8 (2.21 g, 6 mmol) in THF (30 mL) was added 12 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 2 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and acetic acid (343 mL, 6 mmol) was added. The aqueous phase was washed with EtOAc (3x), and then
5 acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO₄. Concentration under reduced pressure gave compound 9 as a solid (1.1 g).

Example 10

10 Compound 10: To a suspension of compound 9 (380 mg, 1.3 mmol) in toluene (2.5 mL) was added thionyl chloride (1 mL, 13 mmol), followed by DMF (1 drop). The mixture was heated at 60°C for 2 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) and CH₂Cl₂ to give a white solid. To the
15 solution of the above solid in CH₂Cl₂ (5 mL) at -20°C was added ethyl lactate (294 µL, 2.6 mmol), followed by pyridine (420 µL, 5.2 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure to give a yellow solid, which was purified by flash column chromatography to generate compound 10 (427 mg).

Example 11

20 Compound 11: To a solution of compound 10 (480 mg) in EtOAc (20 mL) was added 10% Pd-C (80 mg). The reaction mixture was hydrogenated for 6 hrs. The mixture was stirred with celite for 5 mins, and filtered through a pad of celite. Concentration under reduced
25 pressure gave compound 11 (460 mg).

Example 12

Compound 12 was prepared by the methods of the Examples herein

Example 13

30 Compound 13: To a solution of compound 12 (536 mg, 1.0 mmol) in CH₂Cl₂ (10 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 2 hrs, and was concentrated under reduced pressure. The liquid was coevaporated with CH₂Cl₂ (3x) and EtOAc (3x) to give a brown solid. To the solution of above brown solid in acetonitrile (6.5 mL) at 0°C was

added bisfurancarboxylate (295 mg, 1.0 mmol), followed by diisopropylethylamine (350 μ L, 2.0 mmol) and DMAP (24 mg). The mixture was warmed to 25°C, and was stirred for 12 hrs. The mixture was diluted with EtOAc, and was washed sequentially with water (2x), 0.5 N HCl (2x), water (2x), 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over
5 MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 1/1) afford compound 13 (540 mg).

Example 14

Compound 14: To a solution of compound 13 (400 mg, 0.67 mmol) in DMF (3 mL) was
10 added imidazole (143 mg, 2.10 mmol), followed by triethylchlorosilane (224 μ L, 1.34 mmol). The mixture was stirred for 12 hours. The mixture was diluted with EtOAc, and was washed with water (5x) and brine, and dried over MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave a white solid (427 mg). To the solution of
15 above solid in isopropanol (18 mL) was added 20% palladium(II) hydroxide on carbon (120 mg). The mixture was hydrogenated for 12 hours. The mixture was stirred with celite for 5 mins, and filtered through a pad of celite. Concentration under reduced pressure gave compound 14(360 mg).

Example 15

20 Compound 15: To a solution of compound 14 (101 mg, 0.18 mmol) in CH_2Cl_2 (5 mL) was added Dess-Martin periodane (136 mg, 0.36 mmol). The mixture was stirred for 1 hour. Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave compound 15 (98 mg).

Example 16

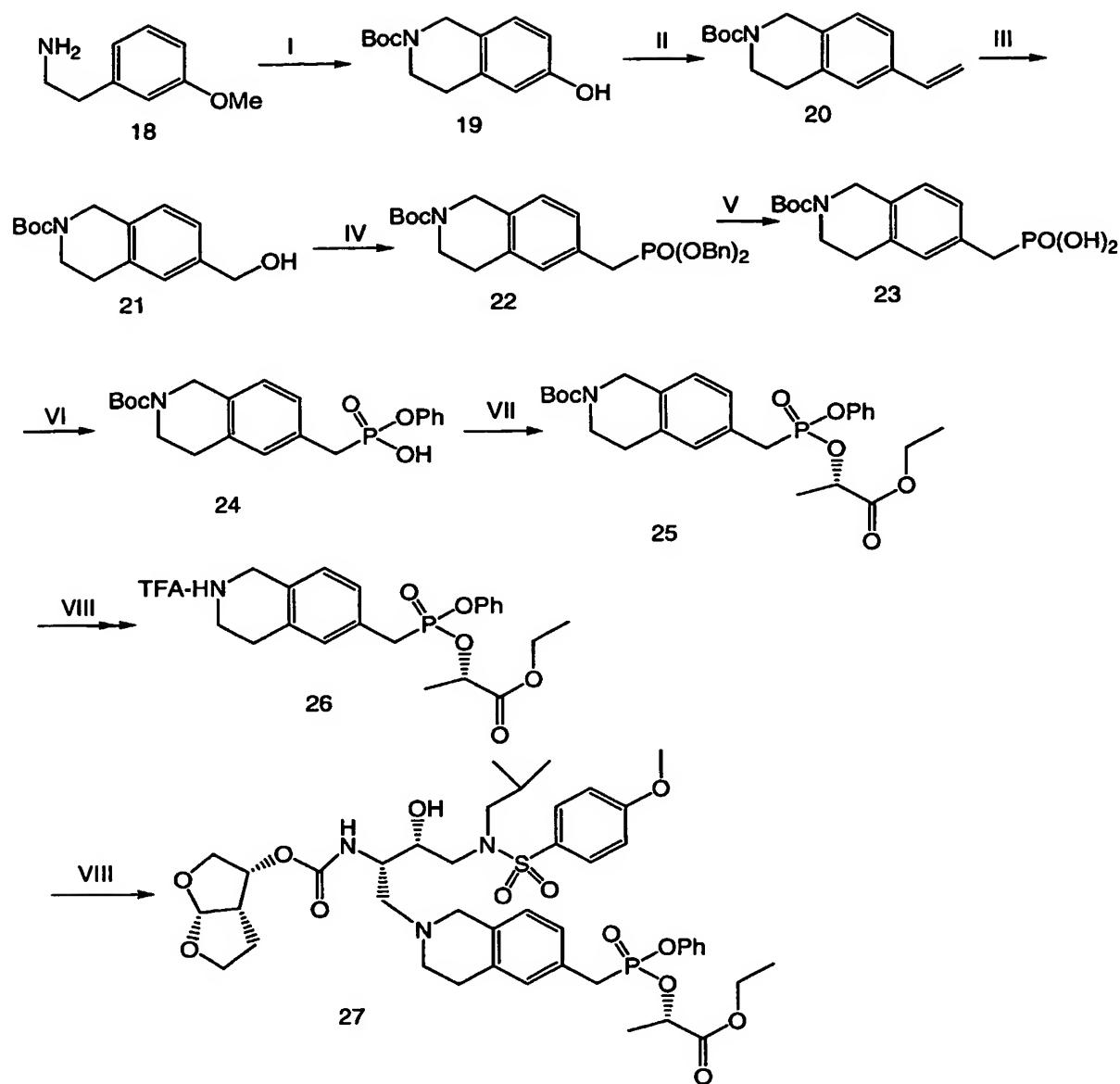
Compound 16: To a solution of compound 15 (50 mg, 0.08 mmol) in EtOAc (0.5 mL) was
25 added compound 11 (150 mg, 0.41 mmol). The mixture was cooled to 0°C, acetic acid (19 μ L, 0.32 mmol) was added, followed by sodium cyanoborohydride (10 mg, 0.16 mmol). The mixture was warmed to 25°C, and was stirred for 14 hrs. The mixture was diluted with
30 EtOAc, and was washed with water (3x) and brine, and was dried over MgSO_4 . Concentration gave a oil. To the solution of above oil in acetonitrile (2.5 mL) was added 48% HF/ CH_3CN (0.1 mL). The mixture was stirred for 30 minutes, and was diluted with EtOAc. The organic phase was washed with water (3x) and brine (1x), and was dried over

MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/3) gave compound 16 (50 mg): ¹H NMR (CDCl₃) δ 7.72 (2 H, d, J = 8.9 Hz), 7.15-7.05 (7 H, m), 7.30 (2 H, d, J = 8.9 Hz), 6.64 (2 H, m), 5.73 (1 H, m), 5.45 (1 H, m), 5.13 (1 H, m), 4.93 (1 H, m), 4.22-3.75 (11 H, m), 3.4 (4 H, m), 3.35-2.80 (5 H, m), 2.1-1.8 (3 H, m), 1.40-1.25 (6 H, m), 0.94 (6 H, m).

Example 17

Compound 17: To a solution of compound 16 (30 mg, 0.04 mmol) in EtOAc (0.8 mL) was added 37% formaldehyde (26 μL, 0.4 mmol). The mixture was cooled to 0°C, acetic acid (20 μL, 0.4 mmol) was added, followed by sodium cyanoborohydride (22 mg, 0.4 mmol). The mixture was warmed to 25°C, and was stirred for 14 hrs. The mixture was diluted with EtOAc, and was washed with water (3x) and brine, and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/3) gave compound 17 (22 mg): ¹H NMR (CDCl₃) δ 7.63 (2 H, m), 7.3-6.9 (9 H, m), 6.79 (2 H, m), 5.68 (1 H, m), 5.2 (1 H, m), 5.10 (1 H, m), 4.95 (1 H, m), 4.22 (2 H, m), 4.2-3.7 (21 H, m), 2.0-1.7 (3 H, m), 1.4-1.2 (6 H, m), 0.93 (6 H, m).

Scheme 5

**Example 18**

Compound 18: Compound 18 was purchased from Aldrich.

5

Example 19

Compound 19: To compound 18 (12.25 g, 81.1 mmol) was added 37% formaldehyde (6.15 mL, 82.7 mmol) slowly. The mixture was heated at 100°C for 1 hour. The mixture was cooled to 25°C, and was diluted with benzene, and was washed with water (2x).

Concentration under reduced pressure gave a yellow oil. To above oil was added 20% HCl (16 mL), and the mixture was heated at 100°C for 12 hours. The mixture was basified with 40% KOH solution at 0°C, and was extracted with EtOAc (3x). The combined organic layer was washed with water and brine, and was dried over MgSO₄. Concentration gave a oil. To the oil was added 48% HBr (320 mL), and the mixture was heated at 120°C for 3 hours.

Water was removed at 100°C under reduced pressure to give a brown solid. To the solution of above solid in water/dioxane (200 mL/200mL) at 0°C was added sodium carbonate (25.7 g, 243 mmol) slowly, followed by di-tert-butyl dicarbonate (19.4 g, 89 mmol). The mixture was warmed to 25°C and stirred for 12 hours. Dioxane was removed under reduced pressure, and the remaining was extracted with EtOAc (3x). The combined organic phase was washed with water (3x) and brine, and was dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 4/1 to 3/1) gave compound 19 as white solid (13.6 g).

Example 20

Compound 20: To a solution of compound 19 (2.49 g, 10 mmol) in CH₂Cl₂ (100 mL) was added N-phenyltrifluoromethanesulfonimide (3.93 g, 11 mmol), followed by cesium carbonate (3.58 g, 11 mmol). The mixture was stirred for 48 hours. The solvent was removed under reduced pressure, and ethyl acetate was added. The reaction mixture was washed with water (3x) and brine (1x), and was dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 6/1) gave a white solid (3.3 g). To the solution of above solid (2.7 g, 7.1 mmol) in DMF (40 mL) was added lithium chloride (2.11 g, 49.7 mmol), followed by dichlorobis(triphenylphosphine) palladium(II) (100 mg, 0.14 mmol).

The mixture was stirred for 3 minutes under high vacuum and recharged with nitrogen. To the above solution was added tributylvinyltin (2.07 mL, 7.1 mmol). The reaction mixture was heated at 90°C for 3 hours and cooled to 25°C. Water was added to the reaction, and the mixture was extracted with ethyl acetate (3X). The combined organic layer was washed with water (6x) and brine, and dried over MgSO₄. Concentration gave an oil. The oil was diluted with CH₂Cl₂ (5 mL), water (128 µL, 7.1mmol) and DBU (1 mL, 7.1 mmol) were added. The mixture was stirred for 5 minutes, and was subjected to flash column chromatography (hexanes/EtOAc = 9/1). Compound 20 was obtained as white solid (1.43 g).

Example 21

Compound 21: To a solution of compound 20 (1.36 g, 5.25 mmol) in ethyl acetate (16 mL) was added water (16 mL), followed by 2.5% osmium tetroxide/tert-butanol (2.63 mL).

- 5 Sodium periodate (2.44 g) was added in portions over 2 minutes period. The mixture was stirred for 45 minutes, and was diluted with ethyl acetate. The organic layer was separated and washed with water (3x) and brine (1x), and dried over MgSO_4 . Concentration gave a brown solid. To the solution of above solid in methanol (100 mL) at 0°C was added sodium borohydride. The mixture was stirred for 1 hour at 0°C , and was quenched with saturated
- 10 NH_4Cl (40 mL). Methanol was removed under reduced pressure, and the remaining was extracted with EtOAc (3x). The combined organic layer was washed with water and brine, and was dried over MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave compound 21 (1.0 g).

15 Example 22

Compound 22: To a solution of compound 21 (657 mg, 2.57 mmol) in CH_2Cl_2 (2 mL) was added a solution of tetrabromocarbon (1.276 g, 3.86 mmol) in CH_2Cl_2 (2 mL). To the above mixture was added a solution of triphenylphosphine (673 mg, 2.57 mmol) in CH_2Cl_2 (2 mL) over 30 minutes period. The mixture was stirred for 2 hours, and was concentrated under

20 reduced pressure. Purification by flash column chromatography (hexanes/EtOAc = 9/1) gave the bromide intermediate (549 mg). To the solution of above bromide (548 mg, 1.69 mmol) in acetonitrile (4.8 mL) was added dibenzyl phosphite (0.48 mL, 2.19 mmol), followed by cesium carbonate (828 mg, 2.54 mmol). The mixture was stirred for 48 hours, and was diluted with EtOAc.

- 25 The mixture was washed with water (3x) and brine, and was dried over MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 3/1 to 100% EtOAc) gave compound 22 (863 mg).

Example 23

- 30 Compound 23: To a solution of compound 22 (840 mg) in ethanol (80 mL) was added 10% palladium on carbon (200 mg). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 23 (504 mg).

Example 24

Compound 24: To a solution of compound 23 (504 mg, 1.54 mmol) in pyridine (10.5 mL) was added phenol (1.45 g, 15.4 mmol), followed by DCC (1.28 g, 6.2 mmol). The mixture was heated at 65°C for 3 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered and washed with EtOAc (2x5 mL). Concentration gave a oil, which was purified by flash column chromatography (CH₂Cl₂/isopropanol = 100/3) to give diphenylphosphonate intermediate (340 mg). To a solution of above compound (341 mg, 0.71 mmol) in THF (1 mL) was added 0.85 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 3 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x), and then acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO₄. Concentration under reduced pressure gave compound 24 as a solid (270 mg).

Example 25:

Compound 25: To a solution of compound 24 (230 mg, 0.57 mmol) in DMF (2 mL) was added ethyl (s)-lactate (130 µL, 1.14 mmol), followed by diisopropylethylamine (400 µL, 2.28 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (504 mg, 1.14 mmol). The mixture was stirred for 14 hours, was diluted with EtOAc. The organic phase was washed with water (5x) and brine (1x), and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/isopropanol = 100/3) gave compound 25 (220 mg).

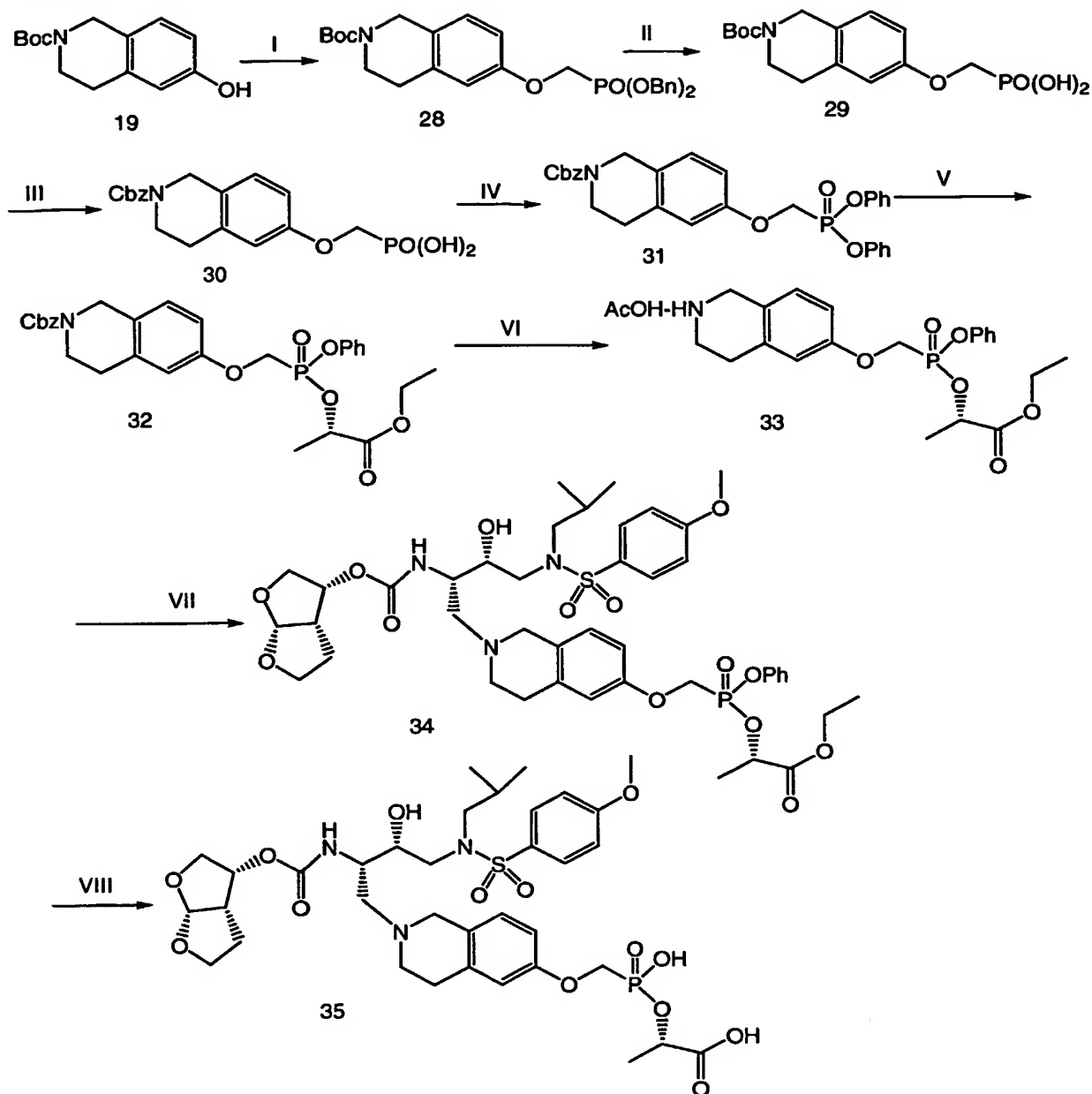
Example 26

Compound 26: To a solution of compound 25 (220 mg) in CH₂Cl₂ (2 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred for 2 hrs, and was concentrated under reduced pressure. The mixture was diluted with EtOAc, and was washed with saturated sodium carbonate solution, water, and brine, and was dried over MgSO₄. Concentration gave compound 26 (170 mg).

Example 27

Compound 27: To a solution of compound 15 (258 mg, 0.42 mmol) in EtOAc (2.6 mL) was added compound 26 (170 mg, 0.42 mmol), followed by acetic acid (75 μ L, 1.26 mmol). The mixture was stirred for 5 minutes, and sodium cyanoborohydride (53 mg, 0.84 mmol) was added. The mixture was stirred for 14 hrs. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/4 to 100/6) gave the intermediate (440 mg). To the solution of above compound (440 mg) in acetonitrile (10 mL) was added 48% HF/ CH₃CN (0.4 mL). The mixture was stirred for 2 hours, and acetonitrile was removed under reduced pressure. The remaining was diluted with EtOAc, and was washed with water (3x) and brine (1x), and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/5) gave compound 27 (120 mg): ¹H NMR (CDCl₃) δ 7.70 (2 H, m), 7.27 (2 H, m), 7.15 (5 H, m), 6.95 (3 H, m), 5.73 (1 H, m), 5.6-5.4 (1 H, m), 5.16 (1 H, m), 4.96 (1 H, m), 4.22-3.60 (13 H, m), 3.42 (2 H, m), 3.4-2.6 (11 H, m), 2.1-3.8 (3 H, m), 1.39 (3 H, m), 1.24 (3 H, m), 0.84 (6 H, m).

Scheme 6



I. $\text{Ti}(\text{OCH}_2\text{PO}(\text{OBn})_2)_2/\text{Cs}_2\text{CO}_3$ II. $\text{H}_2/10\% \text{ Pd-C}$; III. a. $\text{TFA}/\text{CH}_2\text{Cl}_2$;
 b. CbzCl/NaOH ; IV. a. $\text{SOCl}_2/60^\circ\text{C}$; b. $\text{PhOH}/\text{pyridine}$; V. a. NaOH/THF ;
 b. HCl ; c. $\text{SOCl}_2/60^\circ\text{C}$; d. $\text{Ethyl (S) Lactate}/\text{pyridine}$; VI. $\text{H}_2/10\% \text{ Pd-C}/\text{HOAc}$;
 VII. a. compound 15/ $\text{NaBH}_3\text{CN}/\text{HOAc}$; b. $2\% \text{ HF}/\text{CH}_3\text{CN}$;
 VIII. esterase/ $1.0 \text{ PBS buffer}/\text{CH}_3\text{CN}/\text{DMSO}$

Example 28

Compound 28: To a solution of compound 19 (7.5 g, 30 mmol) in acetonitrile (420 mL) was
 5 added dibenzyl triflate (17.8 g, 42 mmol), followed by cesium carbonate (29.4 g, 90 mmol).

The mixture was stirred for 2.5 hours, and was filtered. Acetonitrile was removed under reduced pressure, and the remaining was diluted with EtOAc. The mixture was washed with water (3x) and brine, and was dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 2/1 to 1/1) gave compound 28 (14.3 g).

5

Example 29

Compound 29: To a solution of compound 28 (14.3 g) in ethanol (500 mL) was added 10% palladium on carbon (1.45 g). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 29 (9.1 g).

10

Example 30

Compound 30: To a solution of compound 29 (9.1 g) in CH₂Cl₂ (60 mL) was added trifluoroacetic acid (30 mL). The mixture was stirred for 4 hrs, and was concentrated under reduced pressure. The mixture was coevaporated with CH₂Cl₂ (3x) and toluene, and was dried under high vacuum to give a white solid. The white solid was dissolved in 2.0 N NaOH solution (45 mL, 90 mmol), and was cooled to 0°C. To the above solution was added slowly a solution of benzyl chloroformate (6.4 mL, 45 mmol) in toluene (7 mL). The mixture was warmed to 25°C, and was stirred for 6 hours. 2.0 N sodium hydroxide was added to above solution until pH = 11. The aqueous was extracted with ethyl ether (3x), and was cooled to 0°C. To the above aqueous phase at 0°C was added concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combine organic layers were washed with brine, and were dried over MgSO₄. Concentration gave compound 30 (11.3 g) as a white solid.

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Example 31

Compound 31: To the suspension of compound 30 (11.3 g, 30 mmol) in toluene (150 mL) was added thionyl chloride (13 mL, 180 mmol), followed by DMF (a few drops). The reaction mixture was heated at 65°C for 4.5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x) to afford a brown solid. To the solution of the brown solid in CH₂Cl₂ (120 mL) at 0°C was added phenol (11.28 g, 120 mmol), followed by slow addition of pyridine (14.6 mL, 180 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture

30

was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO₄. Concentration gave a dark oil, which was purified by flash column chromatography (hexanes/EtOAc = 3/1 to 1/1) to afford compound 31 (9.8 g).

5 Example 32

Compound 32: To a solution of compound 31 (9.8 g, 18.5 mmol) in THF (26 mL) was added 20.3 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 2.5 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x). The aqueous phase was cooled to 0°C, and was acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO₄. Concentration under reduced pressure gave a solid (8.2 g). To a suspension of above solid (4.5 g, 10 mmol) in toluene (50 mL) was added thionyl chloride (4.4 mL, 60 mmol), followed by DMF (0.2 mL). The mixture was heated at 70°C for 3.5 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) to give a white solid. To the solution of the above solid in CH₂Cl₂ (40 mL) at 0°C was added ethyl (s)-lactate (2.3 mL, 20 mmol), followed by pyridine (3.2 mL, 40 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was diluted with EtOAc. The organic phase was washed with 1 N HCl, water, and brine, and was dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 2/1 to 1/1) gave compound 32 (4.1 g).

Example 33

Compound 33: To a solution of compound 32 (3.8 g, 6.9 mmol) in EtOAc/EtOH (30 mL/30 mL) was added 10% palladium on carbon (380 mg), followed by acetic acid (400 µL, 6.9 mmol). The mixture was hydrogenated for 3 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 33 (3.5 g).

30 Example 34

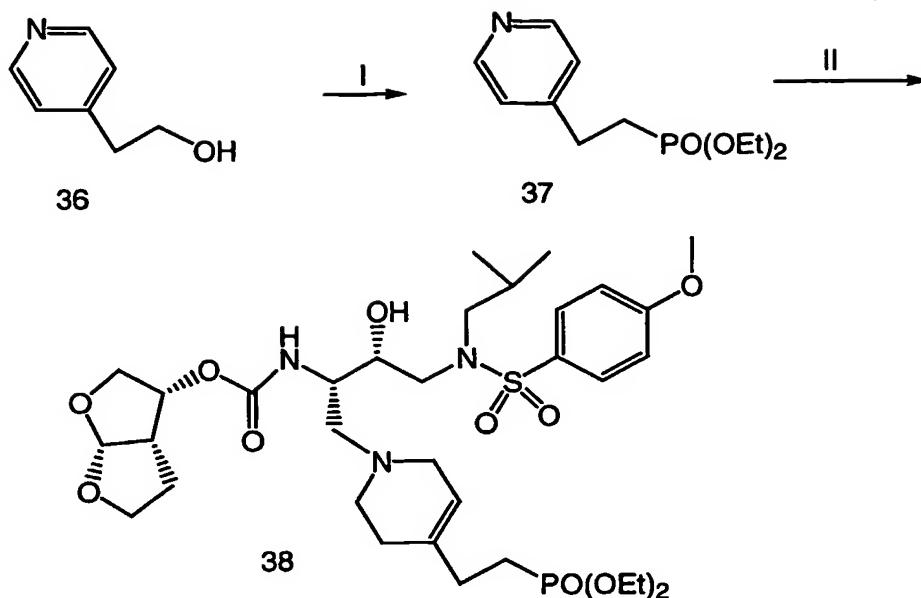
Compound 34: To a solution of compound 15 (1.70 g, 2.76 mmol) in EtOAc (17 mL) was added compound 33 (3.50 g, 6.9 mmol). The mixture was stirred for 5 minutes, and was cooled to 0°C, and sodium cyanoborohydride (347 mg, 5.52 mmol) was added. The mixture

was stirred for 6 hrs. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/6) gave the intermediate (3.4 g). To the solution of above compound (3.4 g) in acetonitrile (100 mL) was added 48% HF/ CH₃CN (4 mL). The mixture was stirred for 2 hours, and acetonitrile was removed under reduced pressure. The remaining was diluted with EtOAc, and was washed with saturated sodium carbonate, water (3x), and brine (1x), and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/5) gave compound 34 (920 mg): ¹H NMR (CDCl₃) δ 7.71 (2 H, m), 7.38-7.19 (5 H, m), 6.92 (3 H, m), 6.75 (2 H, m), 5.73 (1 H, m), 5.57-5.35 (1 H, m), 5.16 (2 H, m), 4.5 (2 H, m), 4.2-3.6 (13 H, m), 3.25-2.50 (11 H, m), 2.0-1.8 (3 H, m), 1.5 (3 H, m), 1.23 (3 H, m), 0.89 (6 H, m).

Example 35

Compound 35: To a solution of compound 34 (40 mg) in CH₃CN /DMSO (1 mL/0.5 mL) was added 1.0 M PBS buffer (5 mL), followed by esterase (200 μL). The mixture was heated at 40°C for 48 hours. The mixture was purified by reverse phase HPLC to give compound 35 (11 mg).

Scheme 7



I. a. SOCl_2 /toluene/60 °C; b. $\text{P}(\text{OEt})_3$ /toluene/120 °C;

II. a. compound 14/ TiF_2O ; b. NaBH_4 /EtOH/HOAc; c. 2% HF/ CH_3CN

Example 36

Compound 36: Compound 36 was purchased from Aldrich.

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Example 37

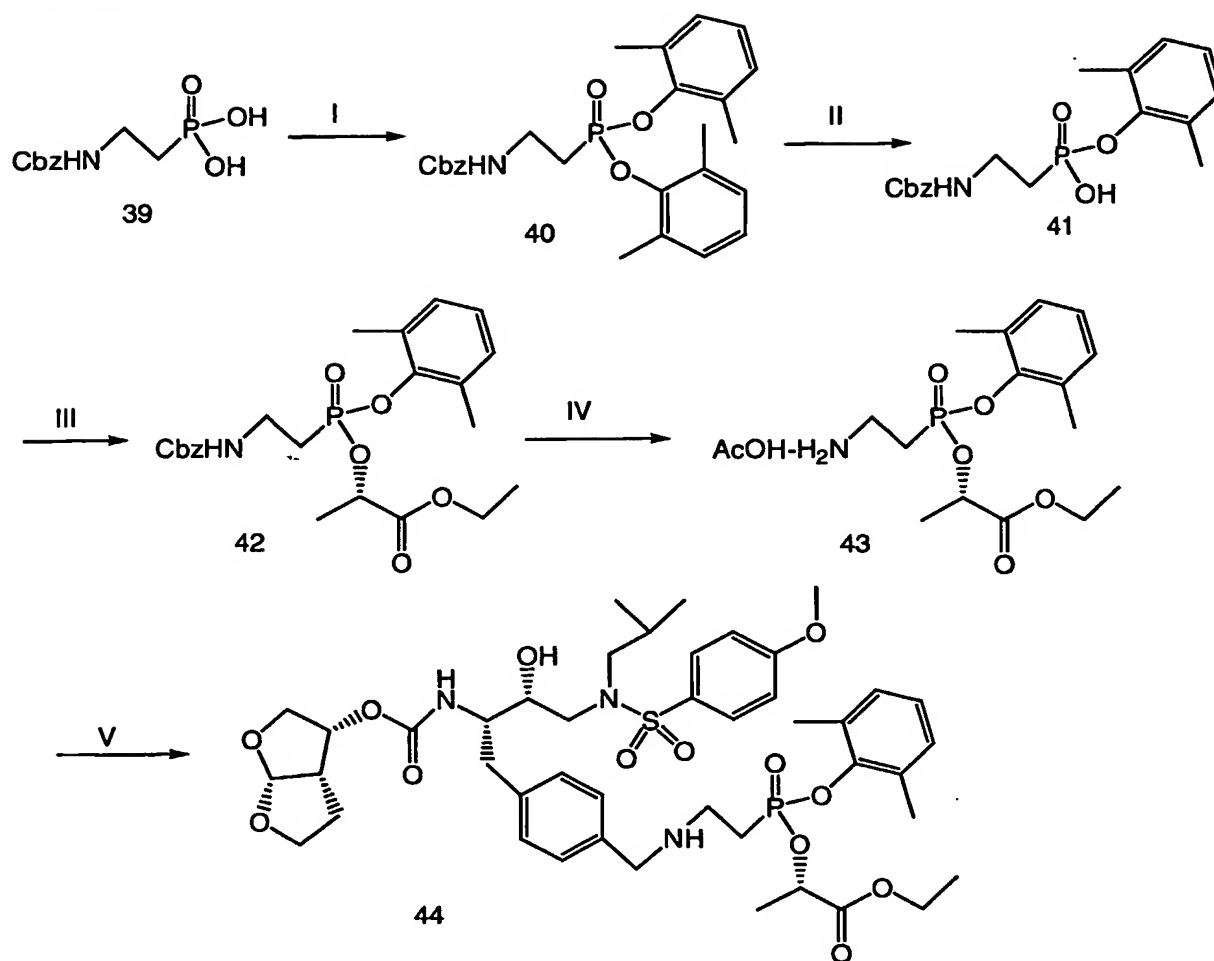
Compound 37: To a solution of compound 36 (5.0 g, 40 mmol) in chloroform (50 mL) was added thionyl chloride (12 mL) slowly. The mixture was heated at 60°C for 2.5 hours. The mixture was concentrated under reduced pressure to give a yellow solid. To the suspension of above solid (5.2 g, 37 mmol) in toluene (250 mL) was added triethyl phosphite (19 mL, 370 mmol). The mixture was heated at 120°C for 4 hours, and was concentrated under reduced pressure to give a brown solid. The solid was dissolved in EtOAc, and was basified with 1.0 N NaOH. The organic phase was separated and was washed with water (2x) and brine, and was dried over MgSO_4 . Purification by flash column chromatography (CH_2Cl_2 /iPrOH = 9/1) gave compound 37 (4.8 g).

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Example 38

Compound 38: To a solution of compound 14 (100 mg, 0.16 mmol) and compound 37 (232 mg, 0.74 mmol) in CH_2Cl_2 (1 mL) at -40°C was added triflic anhydride (40 μL , 0.24 mmol) slowly. The mixture was warmed to 25°C slowly, and was stirred for 12 hours. The mixture
5 was concentrated, and was diluted with EtOH/EtOAc (2 mL/0.4 mL). To the above solution at 0°C was added sodium borohydride (91 mg) in portions. The mixture was stirred at 0°C for 3 hours, and was diluted with EtOAc. The mixture was washed with saturated sodium bicarbonate, water, and brine, and was dried over MgSO_4 . Purification by flash column chromatograph ($\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$ to $100/10$) gave the intermediate (33 mg). To the
10 solution of above intermediate in acetonitrile (2.5 mL) was added 48% HF/ CH_3CN (0.1 mL). The mixture was stirred for 30 minutes, and was diluted with EtOAc. The organic solution was washed with 0.5 N sodium hydroxide, water, and brine, was dried over MgSO_4 . Purification by reverse HPLC gave compound 38 (12 mg): ^1H NMR (CDCl_3) δ 7.72 (2 H, d, $J = 8.9$ Hz), 7.02 (2 H, d, $J = 8.9$ Hz), 5.70 (1 H, m), 5.45 (1 H, m), 5.05 (1 H, m), 4.2-3.4 (19
15 H, m), 3.4-2.8 (5 H, m), 2.45-2.20 (4 H, m), 2.15-1.81 (5 H, m), 1.33 (6 H, m), 0.89 (6 H, m).

Scheme 8



I. a..SOCl₂/toluene/60 C; b. ArOH/pyridine; II. a.NaOH/THF/H₂O; b. HCl;
 III. b.SOCl₂/toluene/60 C; c.ethyl lactate/pyridine; IV. H₂/10%Pd-C/EtOAc/HOAc;
 V. a. compound 6/MgSO₄; b. HOAc/NaCNBH₃

Example 39

Compound 39 was prepared by the methods of the previous Examples.

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Example 40

Compound 40: To the suspension of compound 39 (4.25 g, 16.4 mmol) in toluene (60 mL) was added thionyl chloride (7.2 mL, 99 mmol), followed by DMF (a few drops). The reaction mixture was heated at 65°C for 5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x) to afford a brown solid. To the solution of the brown solid in CH₂Cl₂ (60 ml) at 0°C was added 2,6-dimethylphenol (8.1 g, 66 mmol),

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followed by slow addition of pyridine (8mL, 99 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 3/1 to 1/1)

5 afforded compound 40 (1.38 g).

Example 41

Compound 41: To a solution of compound 40 (1.38 g, 1.96 mmol) in THF (6 mL) was added 3.55 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 24 hours, and THF
10 was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x). The aqueous phase was cooled to 0°C, and was acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO₄. Concentration under reduced pressure gave compound 41 as a white solid (860 mg).

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Example 42

Compound 42: To a suspension of compound 41 (1.00 g, 2.75 mmol) in toluene (15 mL) was added thionyl chloride (1.20 mL, 16.5 mmol), followed by DMF (3 drops). The mixture was heated at 65°C for 5 hours. The solvent and reagent were removed under reduced pressure.
20 The mixture was coevaporated with toluene (2x) to give a brown solid. To the solution of the above solid in CH₂Cl₂ (11 mL) at 0°C was added ethyl (s)-lactate (1.25, 11 mmol), followed by pyridine (1.33 mL, 16.6 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was diluted with EtOAc. The organic phase was washed with 1 N HCl, water, and brine, and was dried over
25 MgSO₄. Purification by flash column chromatography (hexanes/EtOAc = 1.5/1 to 1/1) gave compound 42 (470 mg).

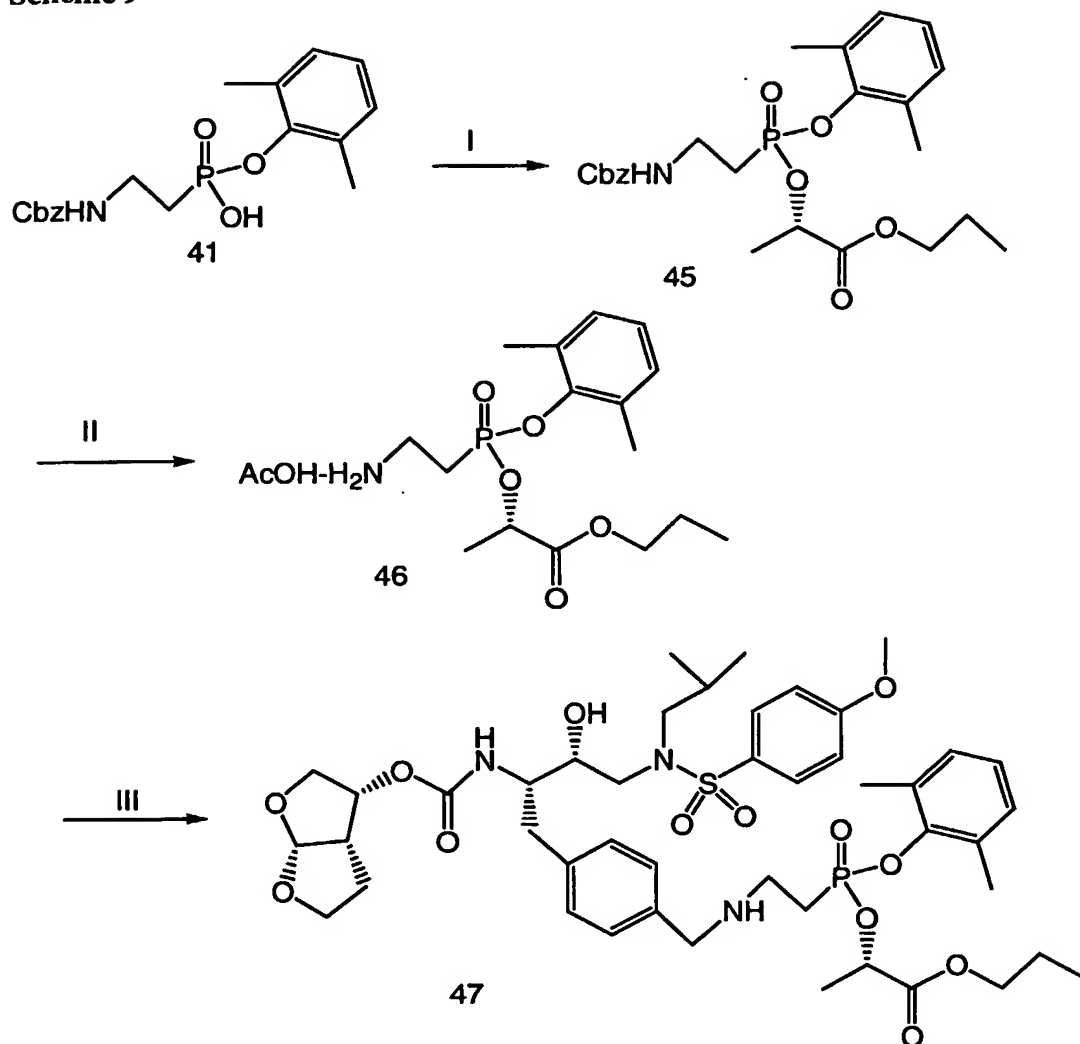
Example 43

Compound 43: To a solution of compound 42 (470 mg) in EtOH (10 mL) was added 10%
30 palladium on carbon (90 mg), followed by acetic acid (150 µL). The mixture was hydrogenated for 6 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 43 (400 mg).

Example 44

Compound 44: To a solution of compound 6 (551 mg, 0.93 mmol) in 1,2-dichloroethane (4 mL) was added compound 43 (400 mg, 1.0 mmol), followed by MgSO_4 (1 g). The mixture was stirred for 3 hours, and acetic acid (148 μL) and sodium cyanoborohydride (117 mg, 1.86 mmol) were added sequentially. The mixture was stirred for 1 hour. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over MgSO_4 . Purification by flash column chromatography (EtOAc to EtOAc/EtOH = 9/1) gave compound 44. Compound 44 was dissolved in CH_2Cl_2 (25 mL), and trifluoroacetic acid (100 μL) was added. The mixture was concentrated to give compound 44 as a TFA salt (560 mg): ^1H NMR (CDCl_3) δ 7.74 (2 H, m), 7.39 (2 H, m), 7.20 (2 H, m), 7.03 (5 H, m), 5.68 (1 H, m), 5.43 (1 H, m), 5.01 (1 H, m), 4.79 (1 H, m), 4.35-4.20 (4 H, m), 4.18-3.4 (11 H, m), 3.2-2.6 (9 H, m), 2.30 (6 H, m), 1.82 (1 H, m), 1.70 (2 H, m), 1.40-1.18 (6 H, m), 0.91 (6 H, m).

Scheme 9



I. b. $SOCl_2$ /toluene/60 C; c. propyl (s)-lactate/pyridine;
 II. H_2 /10%Pd-C/EtOAc/HOAc;
 III. a. compound 6/ $MgSO_4$; b. HOAc/ $NaCNBH_3$

Example 45

Compound 45: To a suspension of compound 41 (863 mg, 2.4 mmol) in toluene (13 mL) was added thionyl chloride (1.0 mL, 14.3 mmol), followed by DMF (3 drops). The mixture was heated at 65°C for 5 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) to give a brown solid. To the solution of the above solid in CH_2Cl_2 (10 mL) at 0°C was added propyl (s)-lactate (1.2 mL, 9.6 mmol), followed by triethylamine (2.0 mL, 14.4 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was

diluted with EtOAc. The organic phase was washed with water and brine, and was dried over MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 1.5/1 to 1/1) gave compound 45 (800 mg).

5 Example 46

Compound 46: To a solution of compound 45 (785 mg) in EtOH (17 mL) was added 10% palladium on carbon (150 mg), followed by acetic acid (250 μL). The mixture was hydrogenated for 16 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 46 (700 mg).

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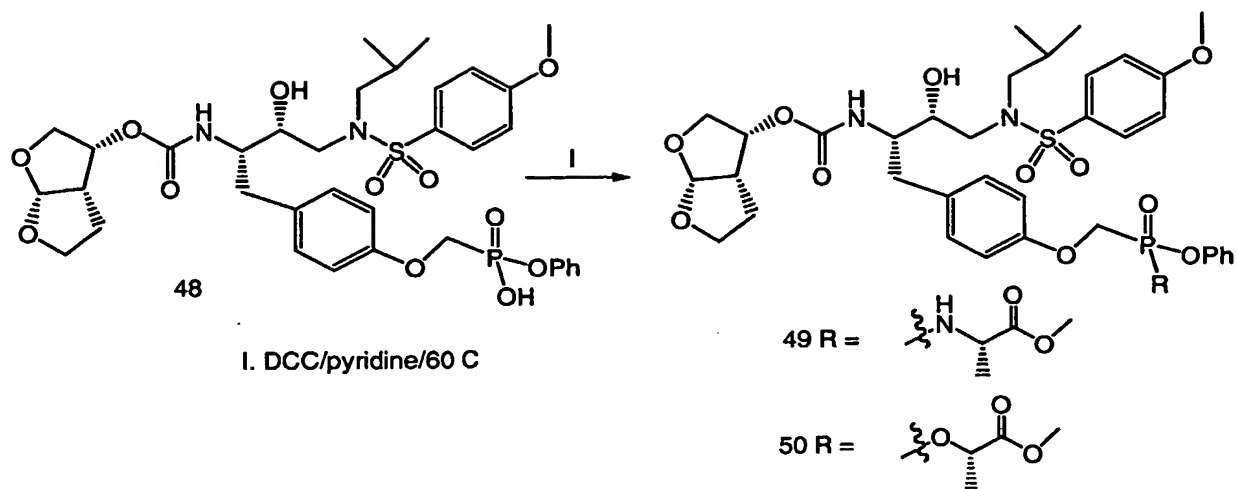
Example 47

Compound 47: To a solution of compound 6 (550 mg, 0.93 mmol) in 1,2-dichloroethane (4 mL) was added compound 43 (404 mg, 1.0 mmol), followed by MgSO_4 (1 g). The mixture was stirred for 3 hours, and acetic acid (148 μL) and sodium cyanoborohydride (117 mg, 1.86 mmol) were added sequentially. The mixture was stirred for 1 hour. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over MgSO_4 . Purification by flash column chromatography (EtOAc to EtOAc/EtOH = 9/1) gave compound 47. Compound 47 was dissolved in CH_2Cl_2 (25 mL), and trifluoroacetic acid (100 μL) was added. The mixture was concentrated to give compound 47 as a TFA salt (650 mg): ^1H NMR (CDCl_3) δ 7.74 (2 H, m), 7.41 (2 H, m), 7.25-7.1 (2 H, m), 7.02 (5 H, m), 5.65 (1 H, m), 5.50 (1 H, m), 5.0-4.75 (2 H, m), 4.25-4.05 (4 H, m), 4.0-3.4 (11 H, m), 3.2-2.6 (9 H, m), 2.31 (6 H, m), 1.82-1.51 (3 H, m), 1.45-1.2 (5 H, m), 0.93 (9 H, m).

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Scheme 10

**Example 48**

Compound 48 was made by the methods of the previous Examples.

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Example 49

Compound 49: To a solution of compound 48 (100 mg, 0.13 mmol) in pyridine (0.75 mL) was added L-alanine methyl ester hydrochloride (73 mg, 0.52 mmol), followed by DCC (161 mg, 0.78 mmol). The mixture was heated at 60°C for 1 hour. The mixture was diluted with EtOAc, and was washed with 0.2 N HCl, water, 5% sodium bicarbonate, and brine, and was dried over MgSO₄. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/5) gave compound 49 (46 mg): ¹H NMR (CDCl₃) δ 7.73 (2 H, m), 7.38-7.18 (7 H, m), 7.03 (2 H, m), 6.89 (2 H, m), 5.68 (1 H, m), 5.05 (1 H, m), 4.95 (1 H, m), 4.30 (3 H, m), 4.0-3.6 (12 H, m), 3.2-2.8 (7 H, m), 1.84-1.60 (3 H, m), 1.38 (3 H, m), 0.93 (6 H, m).

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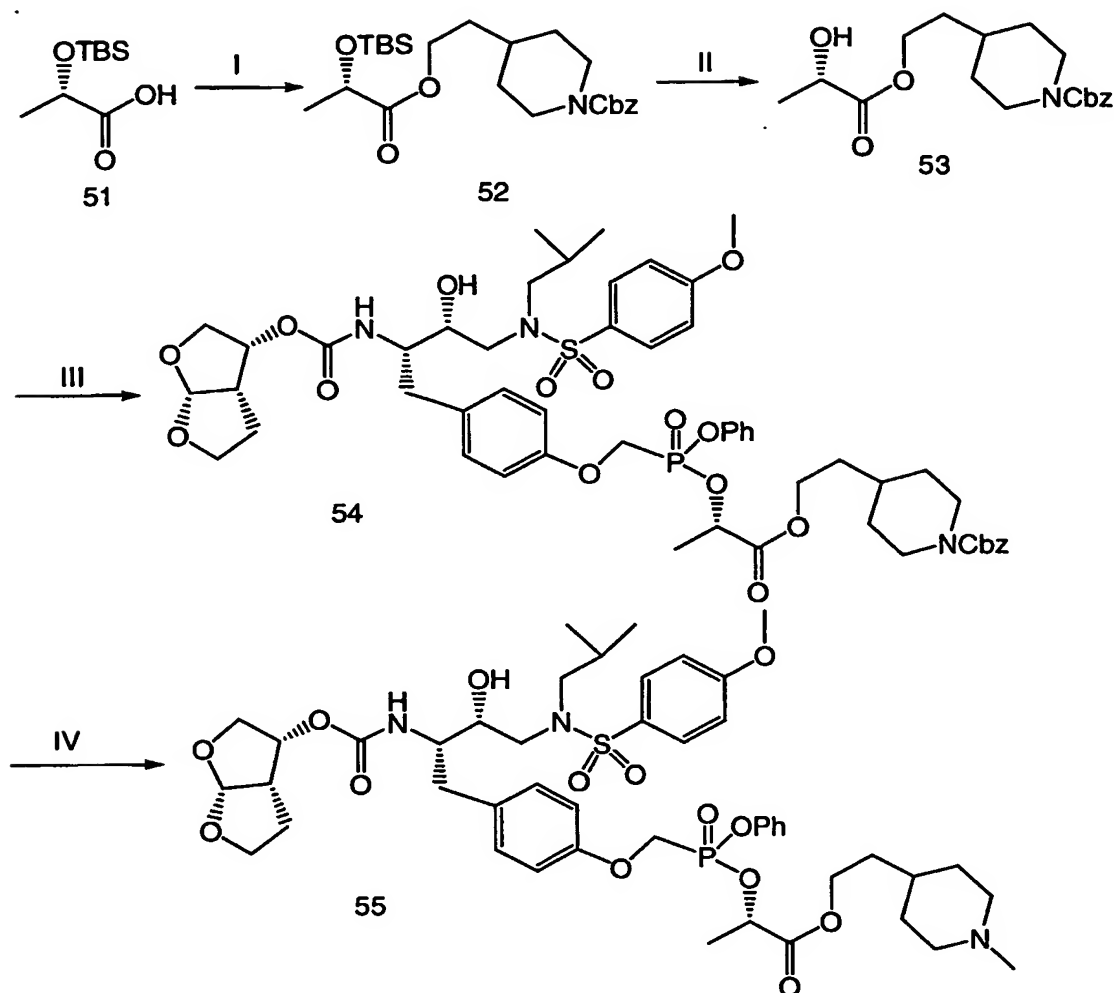
Example 50

Compound 50: To a solution of compound 48 (100 mg, 0.13 mmol) in pyridine (0.75 mL) was added methyl (S)-lactate (41 mg, 0.39 mmol), followed by DCC (81 mg, 0.39 mmol). The mixture was heated at 60°C for 2 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered. Purification by flash column chromatography (CH₂Cl₂/iPrOH = 100/5) gave compound 50 (83 mg): ¹H NMR (CDCl₃) δ 7.74 (2 H, m), 7.38-7.14 (7 H, m), 7.02 (2 H, m), 6.93 (2 H, m), 5.67 (1 H, m),

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m), 5.18 (1 H, m), 5.04 (1 H, m), 4.92 (1 H, m), 4.5 (2 H, m), 4.0-3.68 (12 H, m), 3.2-2.75 (7 H, m), 1.82 (1 H, m), 1.75-1.50 (5 H, m), 0.93 (6 H, m).

Scheme 11



I. Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/ROH/ $i\text{Pr}_2\text{NEt}$;
 II. 15% HF/ CH_3CN ; III. Compound 48/DCC/pyridine/60 °C; IV. a. H_2 /10%Pd-C;
 b. NaBH_3CN /HCHO/HOAc

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Example 51

Compound 51: To a solution of benzyl (S)-lactate (4.0 g, 20 mmol) in DMF (40 mL) was added imidazole (2.7 g, 20 mmol), followed by tert-butyldimethylsilyl chloride (3.3 g, 22 mmol). The mixture was stirred for 14 hours, and diluted with EtOAc. The organic phase was washed with 1.0 N HCl solution (2x), water (2x), and brine (1x), and dried over MgSO_4 .

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Concentration gave the lactate intermediate (6.0 g). To the solution of the above intermediate in EtOAc (200 mL) was added 10% Palladium on carbon (700 mg). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 minutes, and was filtered through a pad of celite. Concentration gave compound 51 (3.8 g).

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Example 52

Compound 52: To a solution of compound 51 (1.55 g, 7.6 mmol) in CH_2Cl_2 (20 mL) was added 4-benzyloxycarbonylpiperidineethanol (2.00 g, 7.6 mmol), followed by benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (4.74 g, 9.1 mmol) and diisopropylethylamine (1.58 mL, 9.1 mmol). The mixture was stirred for 14 hours, and dichloromethane was removed. The mixture was diluted with EtOAc, and was washed with brine, and dried with MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 10/1) gave compound 52 (1.50 g).

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Example 53

Compound 53: To a solution of compound 52 (1.50 g) in CH_3CN was added 58% HF/ CH_3CN (5 mL). The mixture was stirred for 30 minutes, and acetonitrile was removed under reduced pressure. The mixture was diluted with EtOAc, and was washed with water and brine, and was dried over MgSO_4 . Purification by flash column chromatography (hexanes/EtOAc = 1/1) gave compound 53 (1.00 g).

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Example 54

Compound 54: To a solution of compound 48 (769 mg, 1.0 mmol) in pyridine (6.0 mL) was added compound 53 (1.0 g, 3.0 mmol), followed by DCC (618 mg, 3.0 mmol). The mixture was heated at 60°C for 2 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered. Purification by flash column chromatography ($\text{CH}_2\text{Cl}_2/\text{iPrOH}$ = 100/4) gave compound 54 (630 mg).

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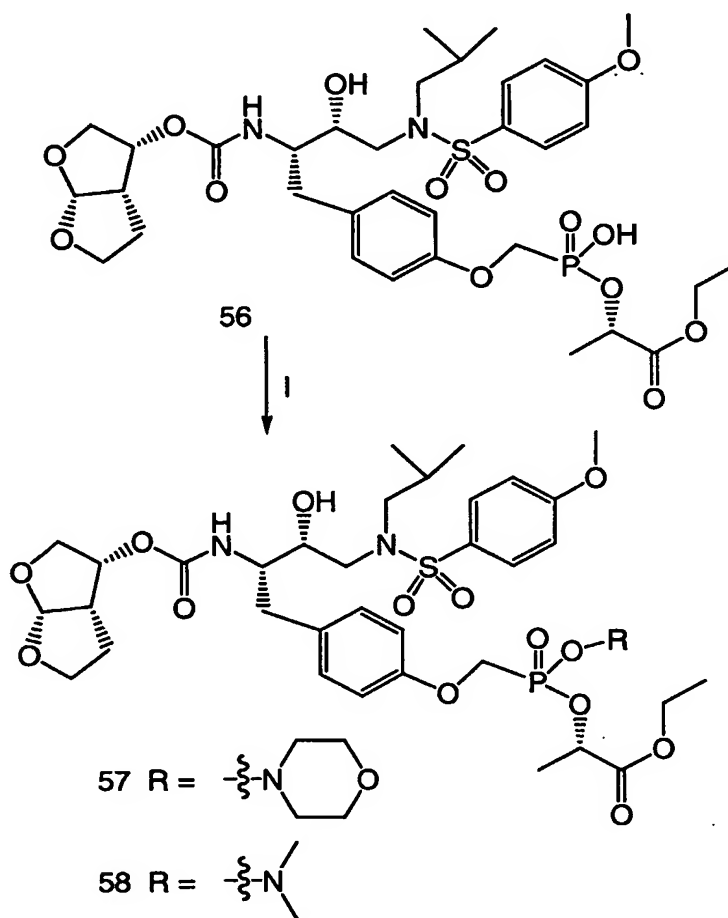
Example 55

Compound 55: To a solution of compound 54 (630 mg, 0.58 mmol) in EtOAc (30 mL) was added 10% Palladium on carbon (63 mg), followed by acetic acid (80 μL). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 minutes, and was filtered through a pad of celite. Concentration gave the intermediate. To the solution of the above

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intermediate in EtOAc (10 mL) was added 37% formaldehyde (88 μ L, 1.18 mmol), followed by acetic acid (101 μ L, 1.77 mmol). The mixture was cooled to 0°C, and sodium cyanoborohydride (74 mg, 1.18 mmol) was added. The mixture was stirred at 25°C for 80 minutes, and was diluted with EtOAc. The mixture was washed with water and brine, and
 5 was dried over MgSO₄. Concentration gave compound 55 as a white solid (530 mg): ¹H NMR (CDCl₃) δ 7.74 (2 H, m), 7.40-7.15 (7 H, m), 7.03 (2 H, m), 6.92 (2 H, m), 5.66 (1 H, m), 5.20-5.00 (3 H, m), 4.58-4.41 (2 H, m), 4.16 (2 H, m), 4.0-3.7 (9 H, m), 3.4-2.6 (14 H, m), 1.90-1.50 (13 H, m), 0.92 (6 H, m).

Scheme 12



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I. R₂NOH/DCC/pyridineExample 56

Compound 56 was made by the methods of the previous Examples.

Example 57

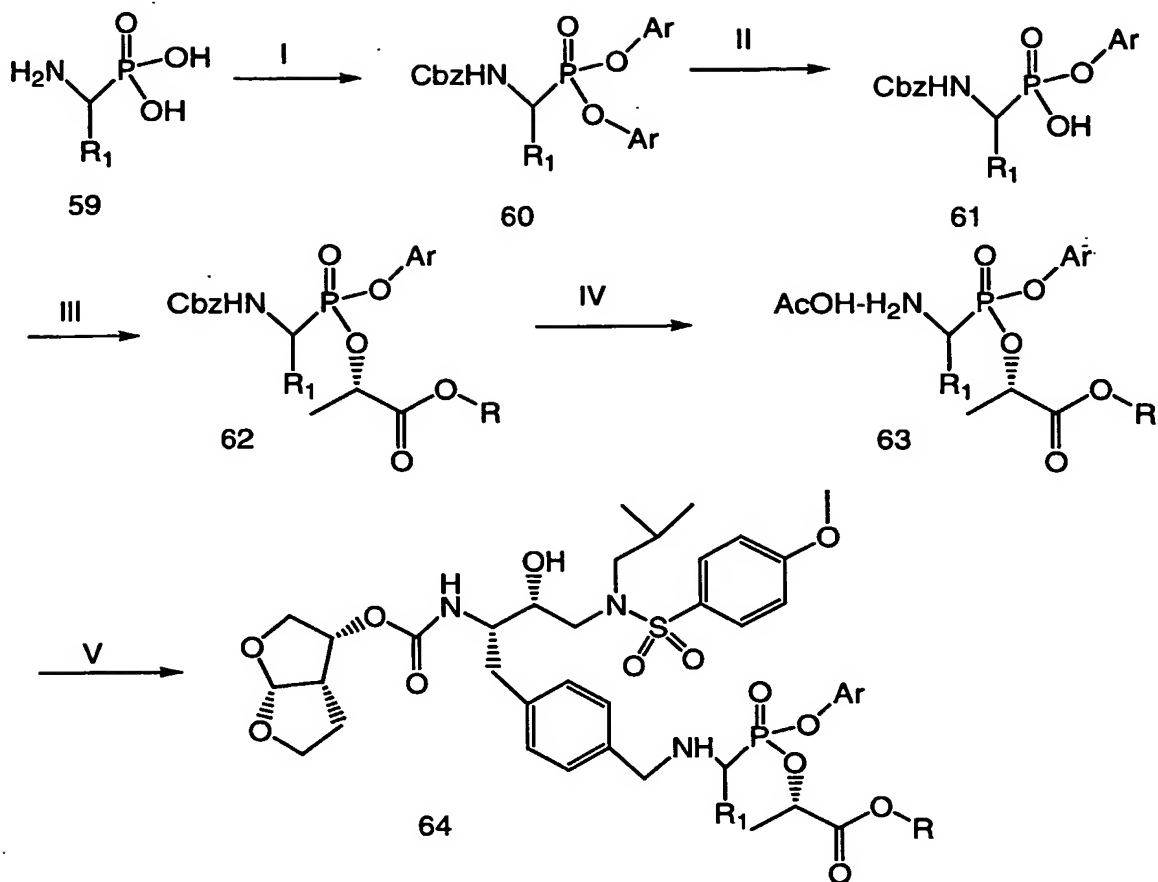
Compound 57: To a solution of compound 56 (100 mg, 0.12 mmol) in pyridine (0.6 mL) was added N-hydroxymorpholine (50 mg, 0.48 mmol), followed by DCC (99 mg, 0.48 mmol).

- 5 The mixture was stirred for 14 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc, and was filtered. Purification by flash column chromatography ($\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$) gave compound 57 (53 mg): ^1H NMR (CDCl_3) δ 7.71 (2 H, d, $J = 8.6$ Hz), 7.15 (2 H, d, $J = 7.6$ Hz), 6.99 (2 H, d, $J = 8.8$ Hz), 6.90 (2 H, m), 5.67 (1 H, m), 5.18 (1 H, m), 5.05 (1 H, m), 4.95 (1 H, m), 4.58-4.38 (2 H, m), 4.21 (2 H, m), 10 4.02-3.80 (13 H, m), 3.55-3.38 (2 H, m), 3.2-2.78 (9 H, m), 1.9-1.8 (1 H, m), 1.8-0.95 (5 H, m), 1.29 (3 H, m), 0.93 (6 H, m).

Example 58

Compound 58: To a solution of compound 56 (100 mg, 0.12 mmol) in pyridine (0.6 mL) was added N,N-dimethylhydroxylamine hydrochloride (47 mg, 0.48 mmol), followed by DCC (99 mg, 0.48 mmol). The mixture was stirred for 6 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc, and was filtered. Purification by flash column chromatography ($\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$) gave compound 58 (35 mg). ^1H NMR (CDCl_3) δ 7.71 (2 H, d, $J = 8.9$ Hz), 7.15 (2 H, d, $J = 8.2$ Hz), 6.99 (2 H, d, $J = 8.4$ Hz), 6.89 (2 H, m), 5.65 (1 H, d, $J = 5.2$ Hz), 5.15 (1 H, m), 4.98 (2 H, m), 4.42 (2 H, m), 4.18 (2 H, m), 20 4.0-3.6 (9 H, m), 3.2-2.7 (13 H, m), 1.92-1.45 (6 H, m), 1.25 (3 H, m), 0.90 (6 H, m).

Scheme 13



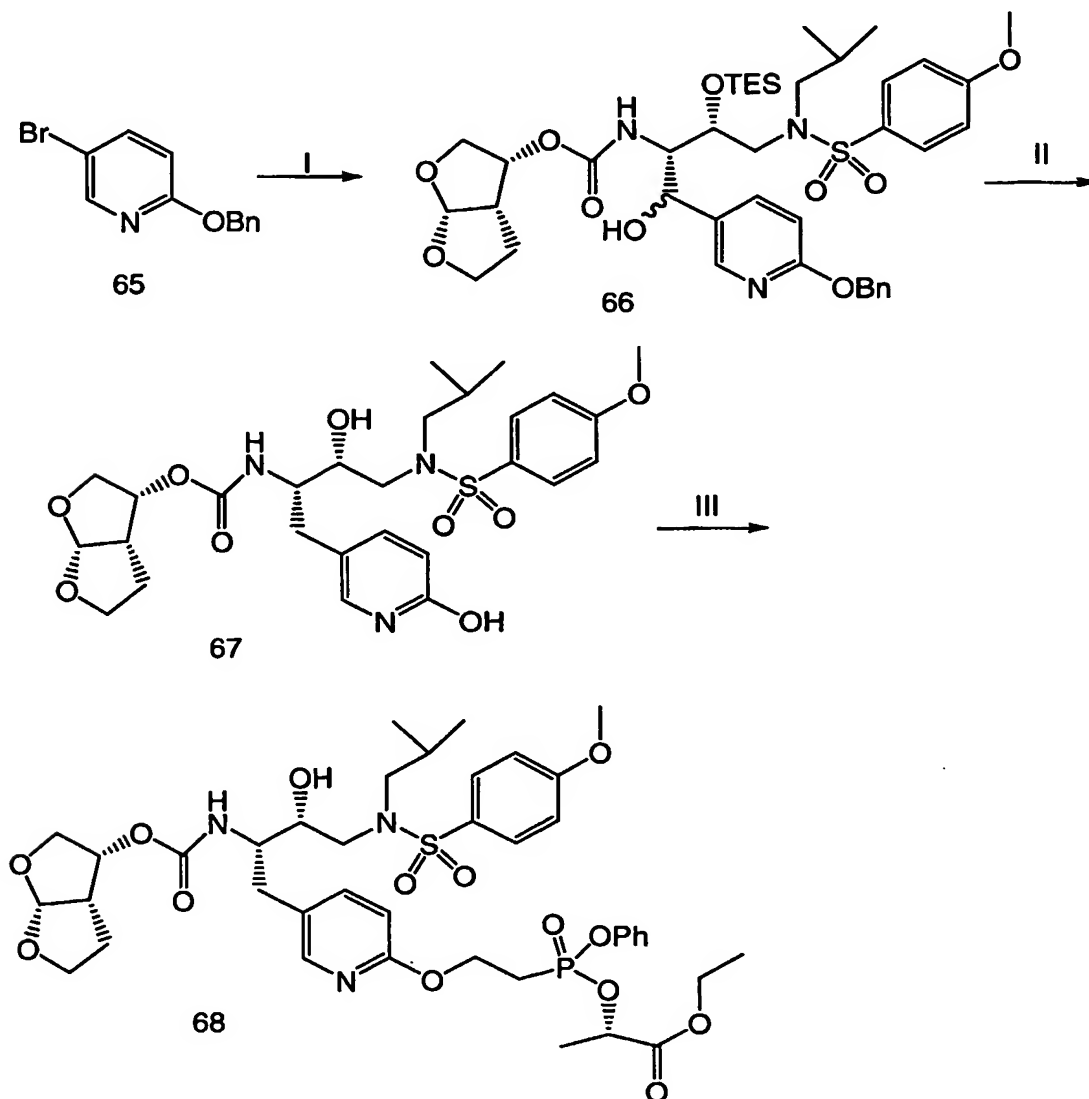
R = Me, Et, Pr, i-Pr; R₁ = H, Me, Et, i-Pr; Ar = phenyl, 2, 6-dimethylphenyl

I. a. CbzCl/NaOH; b. SOCl₂/toluene/60 °C; c. ArOH/pyridine; II. a. NaOH/THF/H₂O; b. HCl;
 III. a. SOCl₂/toluene/60 °C; b. alkyl lactate/pyridine; IV. H₂/10% Pd-C/EtOAc/HOAc;
 V. a. compound 6/MgSO₄; b. HOAc/NaCNBH₃

Aminomethylphosphonic acid 59 is protected as benzyl carbamate. The phosphonic acid is treated with thionyl chloride to generate dichloridate, which reacts with phenol or 2,6-dimethylphenol to give compound 60. Compound 60 is hydrolyzed with sodium hydroxide, followed by acidification to afford monoacid 61. Monoacid 61 is treated with thionyl chloride to generate monochloridate, which reacts with different alkyl (s)-lactates to form compound 62. Compound 62 is hydrogenated with 10% Pd-C in the presence of acetic acid to

give compound 63. Compound 63 reacts with aldehyde 6 in the presence of MgSO_4 to form imine, which is reduced with sodium cyanoborohydride to generate compound 64.

Scheme 14



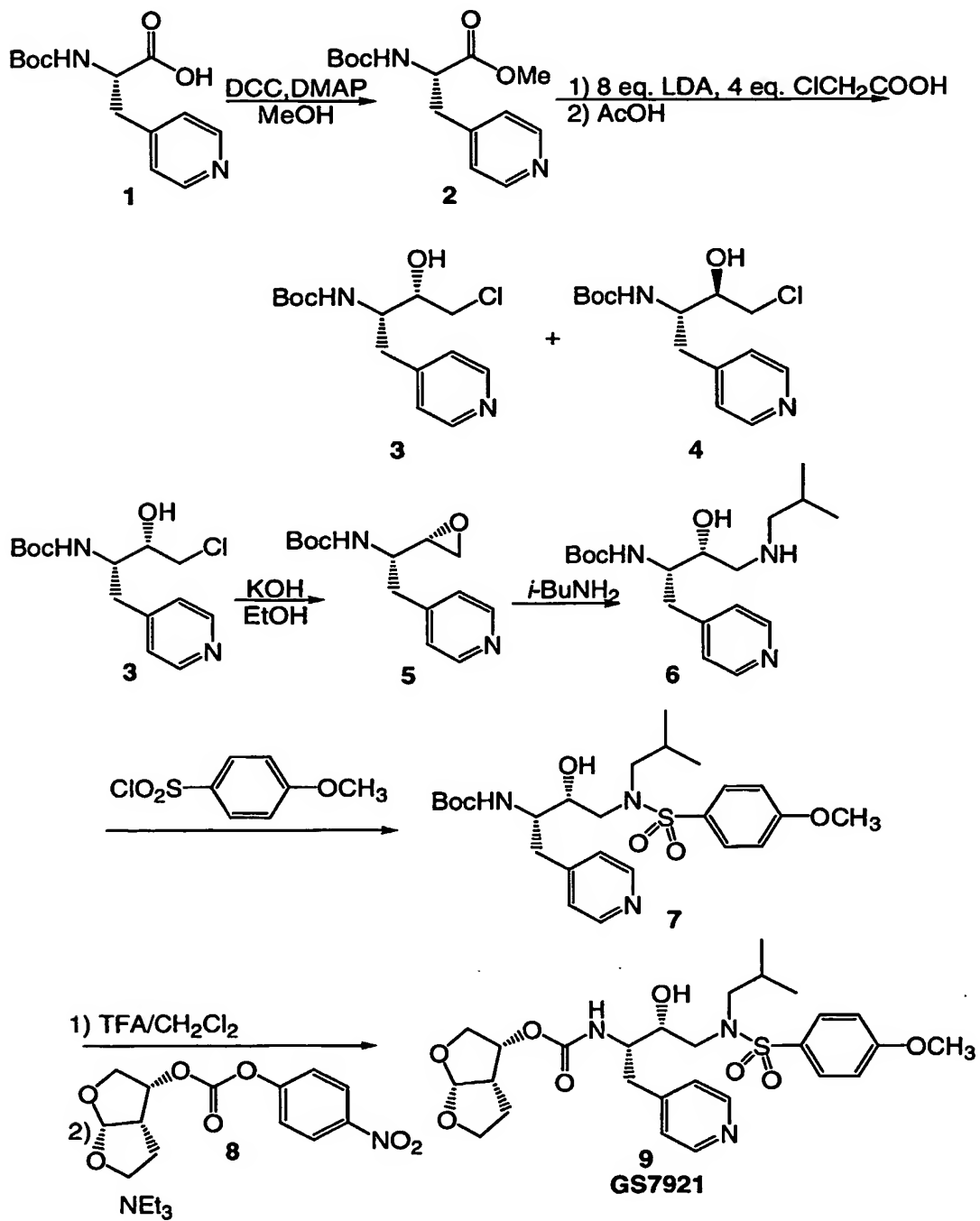
I.a. *n*-BuLi; b. compound 15; II. $\text{H}_2/10\%\text{Pd-C}/\text{HOAc}$; IV. PPh_3/DEAD

- Compound 65 is prepared from 2-hydroxy-5-bromopyridine by alkylation. J. Med. Chem. 1992, 35, 3525. Compound 65 is treated with *n*-Butyl lithium to generate aryl lithium, which reacts with aldehyde 15 to form compound 66. J. Med. Chem. 1994, 37, 3492. Compound 66 is hydrogenated with 10% Pd-C in the presence of acetic acid to give compound 67. J. Med. Chem. 2000, 43, 721. Compound 68 is prepared from compound 67

with corresponding alcohol under Mitsunobu reaction conditions. Bioorg. Med. Chem. Lett. 1999, 9, 2747.

Scheme 1

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Example 1

Methyl 2-(*S*)-(dimethylethoxycarbonylamino)-3-(4-pyridyl)propanoate (2): A solution of N-tert-Butoxycarbonyl-4-pyridylalanine (1, 9.854 g, 37 mmol, Peptech), 4-dimethylaminopyridine (4.52 g, 37 mmol, Aldrich), and dicyclohexylcarbodiimide (15.30 g, 74.2 mmol, Aldrich) in methanol (300 mL) was stirred at 0°C for 2 h and at room temperature for 12 h. After the solids were removed by filtration, the filtrate was concentrated under reduced pressure. More dicyclohexylurea was removed by repeated trituration of the concentrated residue in EtOAc followed by filtration. The residue was chromatographed on silica gel to afford the methyl ester 2 (9.088 g, 88%): ¹H NMR (CDCl₃) δ 8.53 (d, 2H, *J* = 5.7 Hz), 7.09 (d, 2H, *J* = 5.7 Hz), 5.04 (br, 1H), 4.64 (br, 1H), 3.74 (s, 3H), 3.16 (dd, 1H, *J* = 13.5 and 5.7 Hz), 3.02 (dd, 1H, *J* = 13.5 and 6.3 Hz), 1.42 (s, 9H); MS (ESI) 281 (M+H).

Example 2

1-Chloro-3-(*S*)-(dimethylethoxycarbonylamino)-4-(4-pyridyl)-2-(*S*)-butanol (3): A solution of diisopropylamine (37.3 mL, 266 mmol, Aldrich) in THF (135 mL) was stirred at -78°C as a solution of *n*-butyllithium (102 mL of 2.3 M solution and 18 mL of 1.4 M solution 260 mmol, Aldrich) in hexane was added. After 10 min, the cold bath was removed and stirred the solution for 10 min at the ambient temperature. The solution was cooled at -78°C again and stirred as a solution of chloroacetic acid (12.255 g, 130 mmol, Aldrich) in THF (50 mL) was added over 20 min. After the solution was stirred for 15 min, this dianion solution was transferred to a stirred solution of the methyl ester 2 (9.087 g, 32.4 mmol) in THF (100 mL) at 0°C over 15 min. The resulting yellow slurry was stirred at 0°C for 10 min and cooled at -78°C. A solution of acetic acid (29 mL, 507 mmol, Aldrich) in THF (29 mL) was added quickly to the slurry and the resulting slurry was stirred at -78°C for 30 min, at 0°C for 30 min, and at room temperature for 15 min. The resulting slurry was dissolved in saturated NaHCO₃ solution (750 mL) and EtOAc (500 mL). The separated aqueous layer was extracted with EtOAc (300 mL x 2) and the combined organic fractions were washed with water (750 mL x 2) and saturated NaCl solution (250 mL). The resulting solution was dried (MgSO₄) and evaporated under reduced pressure.

A solution of the residue in THF (170 mL) and water (19 mL) was stirred at 0°C as NaBH₄ (3.375 g, 89.2 mmol, Aldrich) was added. After 30 min, the solution was evaporated under reduced pressure and the residue was dissolved in EtOAc, acidified with aqueous NaHSO₄,

and then neutralized by adding saturated aqueous NaHCO₃ solution. The separated aqueous fraction was extracted with EtOAc (100 mL) and the combined organic fractions were washed with water (500 mL) and saturated NaCl solution (100 mL). The solution was dried (MgSO₄) and evaporated under reduced pressure. The residue was chromatographed on silica gel to afford the chlorohydrin 3 and 4 (4.587 g, 47%) as a mixture of two diastereomers (3~4:1). The obtained mixture was recrystallized from EtOAc-hexane twice to obtain pure desired diastereomer 3 (2.444 g, 25%) as yellow crystals: ¹H NMR (CDCl₃) δ 8.53 (d, 2H, *J* = 5.7 Hz), 7.18 (d, 2H, *J* = 5.7 Hz), 4.58 (br, 1H), 3.94 (m, 1H), 3.87 (br, 1H), 3.75-3.54 (m, 2H), 3.05 (dd, 1H, *J* = 13.8 and 3.9 Hz), 2.90 (dd, 1H, *J* = 13.8 and 8.4 Hz), 1.36 (s, 9H); MS (ESI) 301 (M+H).

Example 3

The epoxide 5: A solution of the chlorohydrin 3 (1.171 g, 3.89 mmol) in ethanol (39 mL) was stirred at room temperature as 0.71 M KOH in ethanol (6.6 mL) was added. After 1.5 h, the mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (60 mL) and water (60 mL). The separated aqueous fraction was extracted with EtOAc (60 mL) and the combined organic fractions were washed with saturated NaCl solution, dried (MgSO₄), and concentrated under reduced pressure to obtain the epoxide (1.058 g, quantitative): ¹H NMR (CDCl₃) δ 8.52 (d, 2H, *J* = 6.0 Hz), 7.16 (d, 2H, *J* = 6.0 Hz), 4.57 (d, 1H, *J* = 7.8 Hz), 3.76 (br, 1H), 3.02-2.92 (m, 2H), 2.85-2.79 (m, 2H), 2.78-2.73 (m, 1H), 1.37 (s, 9H); MS (ESI) 265 (M+H).

Example 4

The hydroxy-amine 6: A solution of the epoxide 5 obtained above and *i*-BuNH₂ (3.9 mL, 39.2 mmol, Aldrich) in 58 mL of *i*-PrOH was stirred at 65°C for 2 h and the solution was concentrated under reduced pressure. The residual *i*-PrOH was removed by dissolving the residue in toluene and concentration of the solution twice: ¹H NMR (CDCl₃) δ 8.51 (d, 2H, *J* = 6.0 Hz), 7.18 (d, 2H, *J* = 6.0 Hz), 4.70 (d, 1H, *J* = 9.6 Hz), 3.86 (br, 1H), 3.46 (q, 1H, *J* = 5.8 Hz), 3.06 (dd, 1H, *J* = 14.1 and 3.9 Hz), 2.79 (dd, 1H, *J* = 14.1 and 9.0 Hz), 2.76-2.63 (m, 3H), 2.43 (m, 2H, *J* = 6.9 Hz), 1.73 (m, 1H, *J* = 6.6 Hz), 1.36 (s, 9H), 0.93 (d, 3H, *J* = 6.6 Hz), 0.92 (d, 3H, *J* = 6.6 Hz); MS (ESI) 338 (M+H).

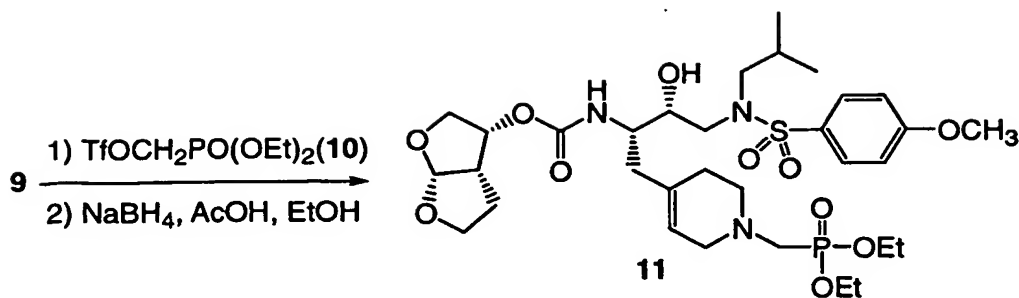
Example 5

The sulfoamide 7: A solution of the crude 6 and *p*-methoxybenzene sulfonyl chloride (890 mg, 4.31 mmol, Aldrich) in CH₂Cl₂ (24 mL) was stirred at 0°C for 2 h and at room temperature for 13 h. The solution was washed with saturated NaHCO₃ solution and the aqueous washing was extracted with CH₂Cl₂ (60 mL). After the combined organic fractions were dried (MgSO₄) and concentrated under reduced pressure, the residue was purified by chromatography on silica gel to obtain the sulfoamide 7 (1.484 g, 75%): ¹H NMR (CDCl₃) δ 8.51 (d, 2H, *J* = 5.7 Hz), 7.73 (d, 2H, *J* = 8.7 Hz), 7.21 (d, 2H, *J* = 5.7 Hz), 7.00 (d, 2H, *J* = 8.7 Hz), 4.68 (d, 1H, *J* = 8.1 Hz), 4.08 (br, 1H), 3.88 (s, 3H), 3.83 (br, 2H), 3.09 (d, 2H, *J* = 5.1 Hz), 3.06-2.80 (m, 4H), 1.85 (m, 1H, *J* = 7.0 Hz), 1.34 (s, 9H), 0.92 (d, 3H, *J* = 6.3 Hz), 0.89 (d, 3H, *J* = 6.6 Hz); MS (ESI) 508 (M+H).

Example 6

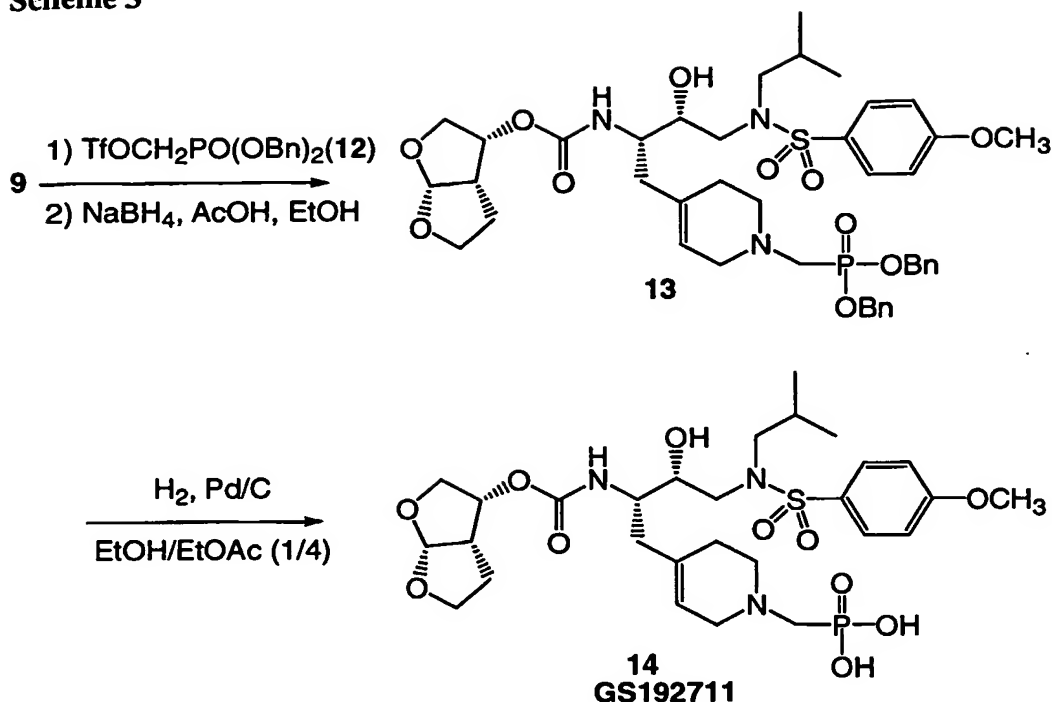
The bisfurancarbamate 9: A solution of the sulfoamide 7 (1.484 g, 2.92 mmol) and trifluoroacetic acid (6.8 mL, 88.3 mmol, Aldrich) in CH₂Cl₂ (18 mL) was stirred at room temperature for 2 h. After the solution was evaporated under reduced pressure, the residue was dissolved in acetonitrile (10 mL) and toluene (10 mL), and evaporated to dryness twice to result crude amine as TFA salt. A solution of the crude amine, dimethylaminopyridine (72 mg, 0.59 mmol, Aldrich), diisopropylethylamine (2.55 mL, 14.6 mmol, Aldrich) in acetonitrile was stirred at 0°C as the bisfurancarboxylic acid 8 (907 mg, 3.07 mmol, obtained from Azar) was added in portion. The solution was stirred at 0°C for 1 h and at room temperature for 19 h, and concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and washed with saturated NaHCO₃ solution (60 mL). After the aqueous washing was extracted with EtOAc (60 mL), the combined organic fractions were washed with saturated NaHCO₃ (60 mL) and saturated NaCl solution (60 mL), dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to obtain the carbamate 9 (1.452 g, 88%): ¹H NMR (CDCl₃) δ 8.50 (d, 2H, *J* = 5.7 Hz), 7.72 (d, 2H, *J* = 8.7 Hz), 7.19 (d, 2H, *J* = 5.7 Hz), 7.01 (d, 2H, *J* = 8.7 Hz), 5.65 (d, 1H, *J* = 5.1 Hz), 5.12 (d, 1H, *J* = 9.3 Hz), 5.02 (q, 1H, *J* = 6.7 Hz), 4.01-3.77 (m, 4H), 3.88 (s, 3H), 3.76-3.63 (m, 2H), 3.18-2.76 (m, 7H), 1.95-1.77 (m, 1H), 1.77-1.56 (m, 2H), 1.56-1.41 (m, 1H), 0.94 (d, 3H, *J* = 6.6 Hz), 0.90 (d, 3H, *J* = 6.9 Hz); MS (ESI) 564 (M+H).

Scheme 2

**GS192710****Example 7**

The tetrahydropyridine-diethyl phosphonate 11: A solution of the pyridine **9** (10.4 mg, 0.018 mmol) and the triflate **10** (8.1 mg, 0.027 mmol, in acetone- d_6 (0.75 mL) was stored at room temperature for 9 h and the solution was concentrated under reduced pressure: ^{31}P NMR (acetone- d_3) δ 14.7; MS (ESI) 714 (M^+). The concentrated crude pyridinium salt was dissolved in ethanol (2 mL) and stirred at room temperature as NaBH_4 (~10 mg, Aldrich) was added occasionally over 4 h. To the mixture was added a solution of acetic acid (0.6 mL, Aldrich) in ethanol (3 mL) until the pH of the mixture became 3~4. More NaBH_4 and acetic acid were added until the reaction was completed. The mixture was carefully concentrated under reduced pressure and the residue was dissolved in saturated NaHCO_3 solution (10 mL). The product was extracted using EtOAc (10 mL x 3) and washed with saturated NaCl solution, dried (MgSO_4), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to obtain the product **11** (8.5 mg, 64%): ^1H NMR (CDCl_3) δ 7.73 (d, 2H, $J = 8.7$ Hz), 7.00 (d, 2H, $J = 8.7$ Hz), 5.71 (d, 1H, $J = 5.1$ Hz), 5.41 (br, 1H), 5.15-5.08 (m, 1H), 5.00 (br, 1H), 4.14 (dq, 4H, $J = 7.2$ Hz), 4.06-3.94 (m, 2H), 3.88 (s, 3H), 3.92-3.80 (m, 2H), 3.75 (dd, 1H, $J = 9.6$ and 6.6 Hz), 3.79-3.61 (m, 1H), 3.24-2.94 (m, 6H), 2.85 (d, 2H, $J = 11.7$ Hz), 2.88-2.76 (m, 2H), 2.75-2.63 (m, 1H), 2.38-2.29 (m, 1H), 2.24-2.2.12 (m, 2H), 2.12-1.78 (m, 4H), 1.30 (t, 6H, $J = 7.1$ Hz), 0.94 (d, 3H, $J = 6.6$ Hz), 0.91 (d, 3H, $J = 6.3$ Hz); ^{31}P NMR (CDCl_3) δ 24.6; MS (ESI) 740 ($\text{M}+\text{Na}$).

Scheme 3



Example 8

5

The tetrahydropyridine-dibenzyl phosphonate 13: The compound 13 was obtained by the same procedure as described for compound 11 using the pyridine 9 (10.0 mg, 0.018 mmol) and the triflate 12 (9.4 mg, 0.022 mmol). The product 13 was purified by preparative TLC to afford the dibenzyl phosphonate 13 (8.8 mg, 59%): ^1H NMR (CDCl_3) δ 7.73 (d, 2H, $J = 8.7$ Hz), 7.35 (s, 10H), 7.00 (d, 2H, $J = 8.7$ Hz), 5.65 (d, 1H, $J = 5.1$ Hz), 5.39 (br, 1H), 5.15-4.92 (m, 6H), 4.03-3.77 (m, 6H), 3.77-3.62 (m, 2H), 3.56 (br, 1H), 3.24-2.62 (m, 9H), 2.32 (d, 1H, $J = 13.5$ Hz), 2.24-1.75 (m, 6H), 0.94 (d, 3H, $J = 6.6$ Hz), 0.89 (d, 3H, $J = 6.3$ Hz); ^{31}P NMR (CDCl_3) δ 25.5; MS (ESI) 842 ($\text{M}+\text{H}$).

10

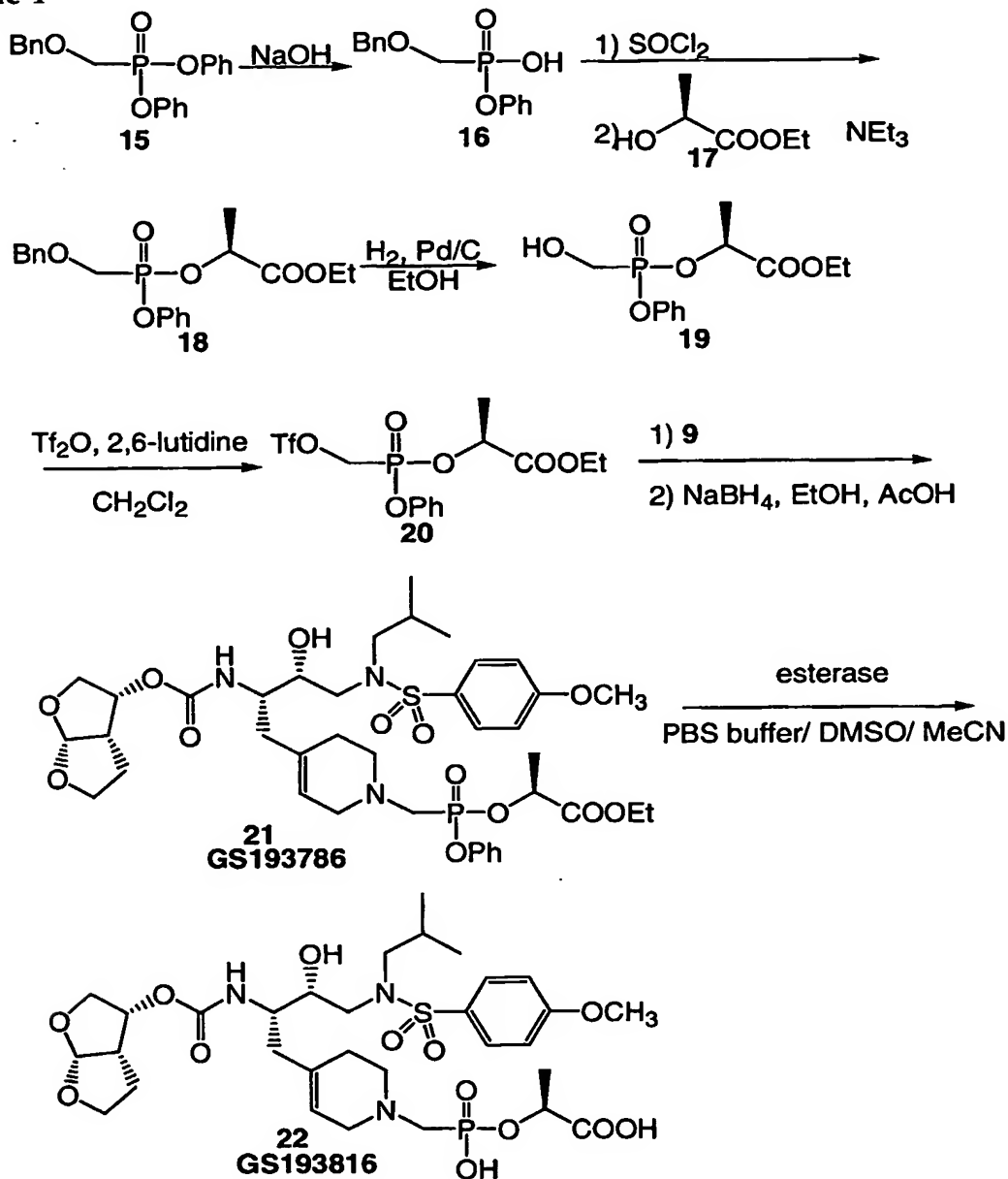
15 Example 9

The phosphonic acid 14: A mixture of the dibenzyl phosphonate 13 (8.8 mg, 0.011 mmol) and 10% Pd/C in EtOAc (2 mL) and EtOH (0.5 mL) was stirred under H_2 atmosphere for 10 h at room temperature. After the mixture was filtered through celite, the filtrate was concentrated to dryness to afford the product 14 (6.7 mg, quantitative): ^1H NMR (CD_3OD) δ 7.76 (d, 2H, $J = 9.0$ Hz), 7.10 (d, 2H, $J = 9.0$ Hz), 5.68 (d, 1H, $J = 5.1$ Hz), 5.49 (br, 1H), 5.11 (m, 1H), 3.90 (s, 3H), 4.04-3.38 (m, 10H), 3.22 (d, 2H, $J = 12.9$ Hz), 3.18-3.00 (m, 2H),

20

2.89-2.75 (m, 2H), 2.68-2.30 (m, 3H), 2.21-1.80 (m, 4H), 0.92 (d, 3H, $J = 6.3$ Hz), 0.85 (d, 3H, $J = 6.3$ Hz); ^{31}P NMR (CD_3OD) δ 6.29; MS (ESI) 662 ($\text{M}+\text{H}$).

Scheme 4



5

Example 10

Diphenyl benzyloxymethylphosphonate 15: To a solution of diphenylphosphite (46.8 g, 200 mmol, Aldrich) in acetonitrile (400 mL) (at ambient temperature) was added potassium carbonate (55.2 g, 400 mmol) followed by the slow addition of benzyl chloromethyl ether (42

mL, 300 mmol, about 60%, Fluka). The mixture was stirred overnight, and was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with water, saturated NaCl, dried (Na_2SO_4), filtered and evaporated. The crude product was chromatographed on silica gel to afford the benzylether (6.8 g, 9.6%) as a colorless liquid.

5

Example 11

Monoacid 16: To a solution of diphenyl benzyloxymethylphosphonate 15 (6.8 g, 19.1 mmol) in THF (100 mL) at room temperature was added 1N NaOH in water (21 mL, 21 mmol). The solution was stirred 3 h. The THF was evaporated under reduced pressure and water (100 mL) was added. The aqueous solution was cooled to 0°C, neutralized to pH 7 with 3N HCl and washed with EtOAc. The aqueous solution was again cooled to 0°C, acidified with 3N HCl to pH 1, saturated with sodium chloride, and extracted with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4), filtered and evaporated, then co-evaporated with toluene to yield the monoacid (4.0 g, 75%) as a colorless liquid. ^1H NMR (CDCl_3) δ 7.28-7.09 (m, 10H), 4.61 (s, 2H), 3.81 (d, 2H); ^{31}P NMR (CDCl_3) δ 20.8.

Example 12

Ethyl lactate phosphonate 18: To a solution of monoacid 16 (2.18 g, 7.86 mmol) in anhydrous acetonitrile (50 mL) under a nitrogen atmosphere was slowly added thionyl chloride (5.7 mL, 78 mmol). The solution was stirred in a 70°C oil bath for three hours, cooled to room temperature and concentrated. The residue was dissolved in anhydrous dichloromethane (50 mL), and this solution cooled to 0°C and stirred under a nitrogen atmosphere. To the stirring solution was added ethyl (S)-(-)-lactate (2.66 mL, 23.5 mmol) and triethylamine (4.28 mL, 31.4 mmol). The solution was warmed to room temperature and allowed to stir for one hour. The solution was diluted with ethyl acetate, washed with water, brine, citric acid and brine again, dried (MgSO_4), filtered through Celite, concentrated under reduced pressure and chromatographed on silica gel using 30% ethylacetate in hexane. The two diastereomers were pooled together. ^1H NMR (CDCl_3) δ 7.40-7.16 (m, 20H), 5.18-5.13 (m, 2H), 4.73 (s, 2H), 4.66 (d, 2H), 4.28-4.11 (m, 5H), 4.05 (d, 2H), 3.95 (d, 2H), 1.62 (d, 3H), 1.46 (d, 3H), 1.30-1.18 (m, 6H); ^{31}P NMR (CDCl_3) δ 19.6, 17.7.

Example 13

Ethyl lactate phosphonate with free alcohol 19: Ethyl lactate phosphonate 18 was dissolved in EtOH (50mL) and under a nitrogen atmosphere 10% Pd-C (approximately 20 wt %) was added. The nitrogen atmosphere was replaced with hydrogen (1atm) and the suspension stirred for two hours. 10% Pd-C was again added (20 wt %) and the suspension stirred five hours longer. Celite was added, the reaction mixture was filtered through Celite and the filtrate was concentrated to afford 1.61 g (71% from monoacid 16) of the alcohol as a colorless liquid. ^1H NMR (CDCl_3) δ 7.40-7.16 (m, 10H), 5.16-5.03 (m, 2H), 4.36-4.00 (m, 8H), 1.62 (d, 3H), 1.46 (d, 3H), 1.30-1.22 (m, 6H); ^{31}P NMR (CDCl_3) δ 22.3, 20.0.

Example 14

Triflate 20: To a solution of ethyl lactate phosphonate with free alcohol 19 (800 mg, 2.79 mmol) in anhydrous dichloromethane (45 mL) chilled to -40°C under a nitrogen atmosphere was added triflic anhydride (0.516 mL, 3.07 mmol) and 2-6 lutidine (0.390 mL, 3.34 mmol). The solution was stirred for 3 hr, then warmed to -20°C and stirred one hour longer. 0.1 equivalents of triflic anhydride and 2-6 lutidine were then added and stirring was resumed for 90 minutes more. The reaction mixture was diluted with ice-cold dichloromethane, washed with ice-cold water, washed with ice-cold brine and the organic layer was dried (MgSO_4) and filtered. The filtrate was concentrated and chromatographed on silica gel using 30% EtOAc in hexane as eluent to afford 602 mg (51%) of the triflate diastereomers as a slightly pink, transparent liquid. ^1H NMR (CDCl_3) δ 7.45-7.31 (m, 4H), 7.31-7.19 (m, 6H), 5.15-4.75 (m, 6H), 4.32-4.10 (4H), 1.62 (d, 3H), 1.50 (d, 3H), 1.30-1.22 (m, 6H); ^{31}P NMR (CDCl_3) δ 10.3, 8.3.

Example 15

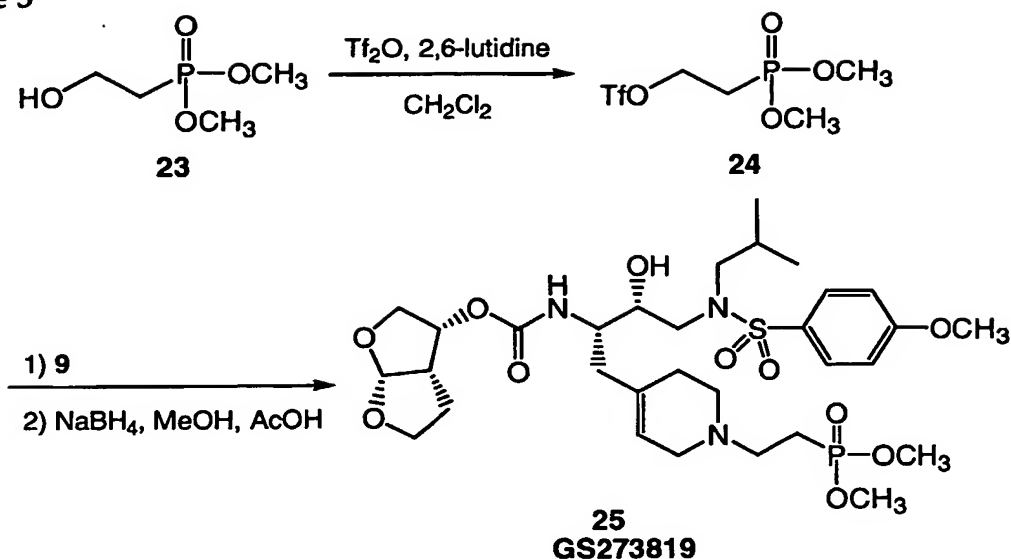
The tetrahydropyridine-prodrug 21: A solution of the pyridine 9 (11.1 mg, 0.020 mmol) and the triflate 20 (11.4 mg, 0.027 mmol) in acetone- d_6 (0.67 mL, Aldrich) was stored at room temperature for 7 h and the solution was concentrated under reduced pressure: ^{31}P NMR (acetone- d_6) δ 11.7, 10.9; MS (ESI) 838 (M+H). The concentrated crude pyridinium salt was dissolved in ethanol (1 mL) and added 2~3 drops of a solution of acetic acid (0.6 mL, Aldrich) in ethanol (3 mL). The solution was stirred at 0°C as NaBH_4 (7~8 mg, Aldrich) was

added. More acetic acid solution was added to adjust pH 3~4 of the reaction mixture. Additions of NaBH₄ and the acetic acid solution were repeated until the reaction was completed. The mixture was carefully concentrated under reduced pressure and the residue was purified by chromatography on C18 reverse phase column material followed by
5 preparative TLC using C18 reverse phase plate to obtain the prodrug **21** (13.6 mg, 70%) as a 2:3 mixture of two diastereomers: ¹H NMR (CD₃CN) δ 7.78 (d, 2H, *J* = 9.0 Hz), 7.48-7.42 (m, 2H), 7.35-7.27 (m, 3H), 7.10 (d, 2H, *J* = 9.0 Hz), 5.86 (m, 1H), 5.60 (m, 1H), 5.48 (br, 1H), 5.14-5.03 (m, 2H), 4.29-4.13 (m, 2H), 3.89 (s, 3H), 3.97-3.32 (m, 12H), 3.29 (br, 0.4H), 3.24 (br, 0.6H), 3.02-2.82 (m, 4H), 2.64-2.26 (m, 3H), 2.26-2.08 (m, 1H), 1.94-1.76 (m, 3H),
10 1.57 (d, 1.8H, *J* = 6.9 Hz), 1.46 (d, 1.2H, *J* = 6.9 Hz), 1.28 (d, 1.2H, *J* = 6.9 Hz), 1.21 (d, 1.8H, *J* = 7.2 Hz), 0.92-0.88 (m, 6H); ³¹P NMR (CD₃CN) δ 14.4 (0.4P), 13.7 (0.6P); MS (ESI) 838 (M+H).

Example 16

15 Metabolite **22**: To a solution of the prodrug **21** (10.3 mg, 0.011 mmol) in DMSO (0.1 mL) and acetonitrile (0.2 mL) was added 0.1 M PBS buffer (3 mL) mixed thoroughly to result a suspension. To the suspension was added porcine liver esterase suspension (0.05 mL, EC3.1.1.1, Sigma). After the suspension was stored in 37°C for 1.5 h, the mixture was
20 centrifuged and the supernatant was taken. The product was purified by HPLC and the collected fraction was lyophilized to result the product **22** as trifluoroacetic acid salt (7.9 mg, 86%): ¹H NMR (D₂O) δ 7.70 (d, 1H), 7.05 (d, 2H), 5.66 (d, 1H), 5.40 (br, 1H), 5.02 (br, 1H), 4.70 (br, 1H), 3.99-3.89 (m, 2H), 3.81 (s, 3H), 3.83-3.50 (m, 8H), 3.34-2.80 (m, 7H), 2.50-2.18 (m, 3H), 2.03 (m, 1H), 1.92-1.70 (m, 3H), 1.39 (d, 3H), 0.94 (d, 3H), 0.93 (d, 3H);
25 ³¹P NMR (D₂O) δ 9.0, 8.8; MS (ESI) 734 (M+H).

Scheme 5

Example 17

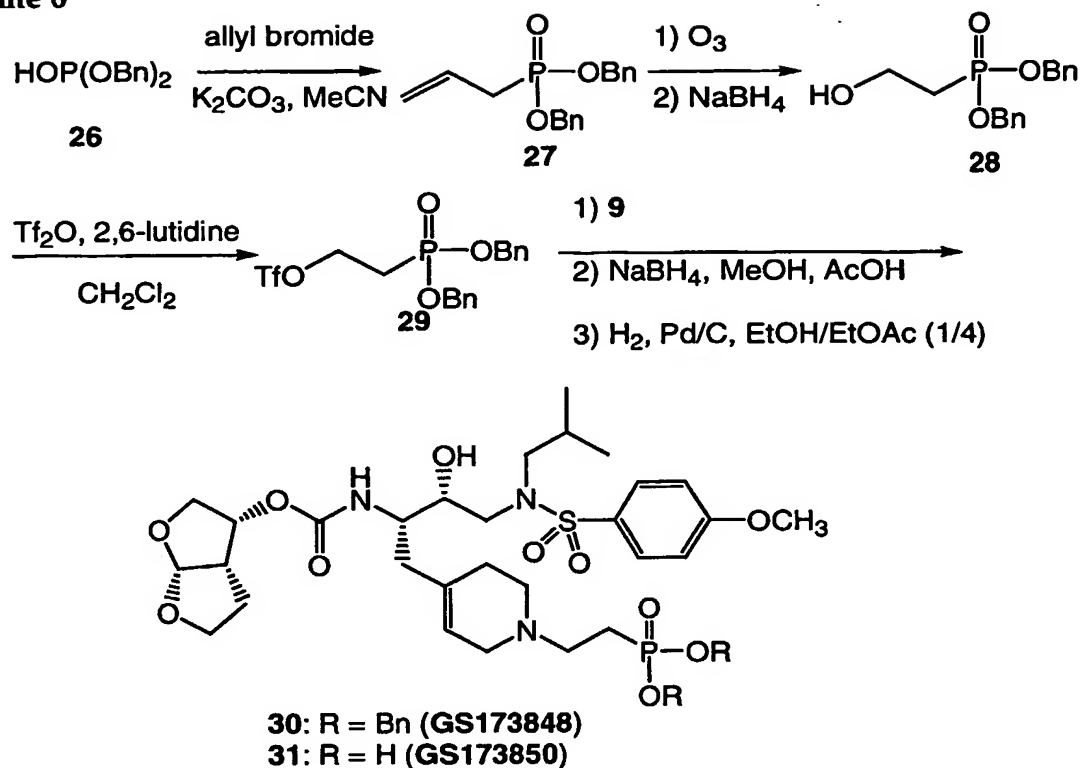
5 **Triflate 24:** Triflate **24** was prepared analogously to triflate **20**, except that dimethylhydroxyethylphosphonate **23** (Aldrich) was substituted for ethyl lactate phosphonate with free alcohol **19**.

10 Example 18

Tetrahydropyridine 25: Tetrahydropyridine **25** was prepared analogously to tetrahydropyridine **30**, except that triflate **24** was substituted for triflate **29**.

^1H NMR (CDCl_3) δ 7.71 (d, 2H), 7.01 (d, 2H), 5.71 (d, 2H), 5.43 (bs, 1H), 5.07-4.87 (m, 1H), 4.16-3.46 (m, 13H), 3.34-3.18 (m, 3H), 3.16-2.80 (m, 5H), 2.52-1.80 (m, 12H), 1.28-1.04 (m, 3H+ H_2O peak), 0.98-0.68 (m, 6H).

Scheme 6

5 Example 19

Dibenzylyl phosphonate with double bond 27: To a stirring solution of allyl bromide (4.15 g, 34 mmol, Aldrich) and dibenzylphosphite (6 g, 23 mmol, Aldrich) in acetonitrile (25 mL) was added potassium carbonate (6.3 g, 46 mmol, powder 325 mesh Aldrich) to create a suspension, which was heated to 65°C and stirred for 72 hours. The suspension was cooled to room temperature, diluted with ethyl acetate, filtered, and the filtrate was washed with water, then brine, dried (MgSO₄), concentrated and used directly in the next step.

Example 20

Dibenzylylhydroxyethylphosphonate 28: Dibenzylyl phosphonate with double bond 27 was dissolved in methanol (50mL), chilled to -78°C, stirred, and subjected to ozone by bubbling ozone into the solution for three hours until the solution turned pale blue. The ozone flow was stopped and oxygen bubbling was done for 15 minutes until the solution became colorless. Sodium borohydride (5 g, excess) was added slowly portionwise. After the evolution of gas subsided the solution was allowed to warm to room temperature, concentrated, diluted with ethyl acetate, made acidic with acetic acid and water and

partitioned. The ethyl acetate layer was washed with water, then brine and dried (MgSO_4), filtered, concentrated and chromatographed on silica gel eluting with a gradient of eluent from 50% ethyl acetate in hexane to 100% ethyl acetate, affording 2.76 g of the desired product. ^1H NMR (CDCl_3) δ 7.36 (m, 10H), 5.16–4.95 (m, 4H), 3.94–3.80 (dt, 2H), 2.13–2.01 (dt, 2H); ^{31}P NMR (CDCl_3) δ 31.6.

Example 21

Dibenzyl phosphonate 30: A solution of the alcohol 28 (53.3 mg, 0.174 mmol) and 2,6-lutidine (0.025 mL, 0.215 mmol, Aldrich) in CH_2Cl_2 (1 mL) was stirred at -45°C as trifluoromethanesulfonic anhydride (0.029 mL, 0.172 mmol, Aldrich) was added. The solution was stirred for 1 h at -45°C and evaporated under reduced pressure to obtain the crude triflate 29.

A solution of the crude triflate 29, 2,6-lutidine (0.025 mL, 0.215 mmol, Aldrich), and the pyridine 9 in acetone- d_6 (1.5 mL, Aldrich) was stored at room temperature for 2 h. The solution was concentrated under reduced pressure to obtain crude pyridinium product: ^{31}P NMR (acetone- d_6) δ 25.8; MS (ESI) 852 (M^+).

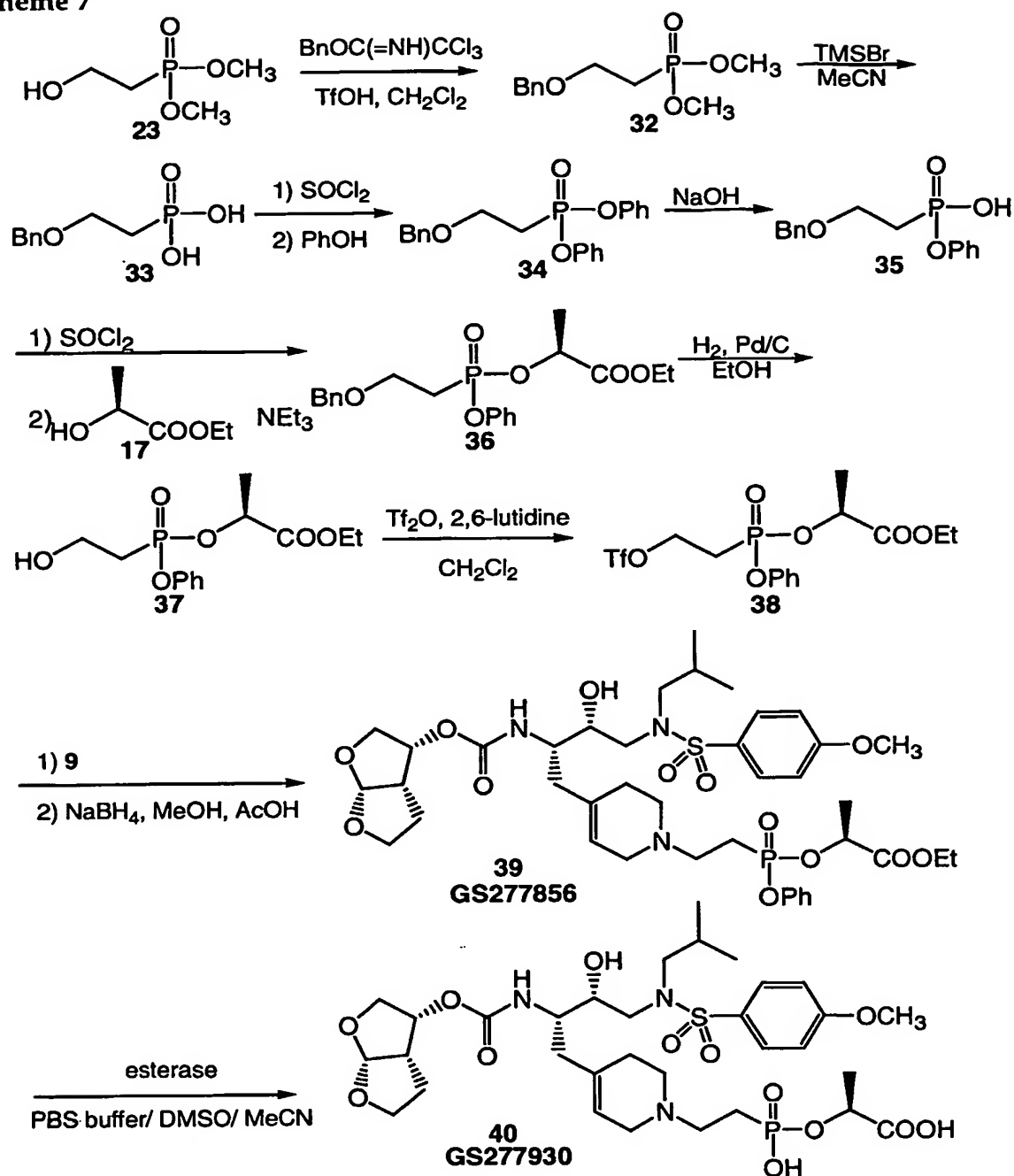
To a solution of the crude pyridinium salt in ethanol (2 mL) was added 7–8 drops of a solution of acetic acid (0.4 mL, Aldrich) in ethanol (2 mL). The solution was stirred at 0°C as NaBH_4 (7–8 mg) was added. The solution was maintained to be pH 3–4 by adding the acetic acid solution. More NaBH_4 and the acetic acid were added until the reduction was completed. After 4 h, the mixture was concentrated and the remaining residue was dissolved in saturated NaHCO_3 (10 mL). The product was extracted with EtOAc (10 mL x 3), dried (MgSO_4), and concentrated under reduced pressure. The residue was purified by repeated chromatography on silica gel followed by HPLC purification. Lyophilization of the collected fraction resulted the product 30 (13.5 mg, 26%) as trifluoroacetic acid salt: ^1H NMR (CDCl_3) δ 7.72 (d, 2H, $J = 8.7$ Hz), 7.36 (br, 10H), 7.00 (d, 2H, $J = 8.7$ Hz), 5.69 (d, 1H, $J = 5.1$ Hz), 5.41 (br, 1H), 5.13–4.93 (m, 6H), 4.05–2.5 (m, 19H), 3.88 (s, 3H), 2.5–1.9 (m, 5H), 1.90–1.74 (m, 2H), 0.88 (d, 6H, $J = 6.1$ Hz); ^{31}P NMR (CDCl_3) δ 25.8; MS (ESI) 856 ($\text{M}+\text{H}$).

Example 22

Phosphonic acid 31: A mixture of the dibenzyl phosphonate 30 (9.0 mg, 0.009 mmol) and 10% Pd/C (5.2 mg, Aldrich) in EtOAc (2 mL) and ethanol (0.5 mL) was stirred under H_2 atmosphere for 3 h at room temperature. After the mixture was filtered through celite, a drop

of trifluoroacetic acid (Aldrich) was added to the filtrate and the filtrate was concentrated to dryness to afford the product **31** (6.3 mg, 86%): ^1H NMR (CD_3OD) δ 7.76 (d, 2H, $J = 9.0$ Hz), 7.11 (d, 2H, $J = 9.0$ Hz), 5.69 (d, 1H, $J = 5.1$ Hz), 5.54 (br, 1H), 5.09 (br, 1H), 4.05-3.84 (m, 4H), 3.89 (s, 3H), 3.84-3.38 (m, 9H), 3.07 (dd, 2H, $J = 13.5$ and 8.4 Hz), 2.9-2.31 (m, 5H), 2.31-1.83 (m, 6H), 0.92 (d, 3H, $J = 6.3$ Hz), 0.85 (d, 3H, $J = 6.9$ Hz); ^{31}P NMR (CD_3OD) δ 21.6; MS (ESI) 676 ($\text{M}+\text{H}$).

Scheme 7

5 Example 23

Benzylether 32: A solution of dimethyl hydroxyethylphosphonate (5.0 g, 32.5 mmol, Across) and benzyl 2,2,2-trichloroacetimidate (97.24 mL, 39.0 mmol, Aldrich) in CH_2Cl_2 (100 mL) at 0°C under a nitrogen atmosphere was treated with trifluoromethanesulfonic acid (0.40 mL). Stirring was performed for three hours at 0°C and the reaction was then allowed to warm to

room temperature while stirring continued. The reaction continued for 15 hours, and the reaction mixture was then diluted with dichloromethane, washed with saturated sodium bicarbonate, washed with brine, dried (MgSO_4), concentrated under reduced pressure and chromatographed on silica gel eluting with a gradient of eluent from 60% EtOAc in hexane to 100% EtOAc to afford 4.5 g, (57%) of the benzyl ether as a colorless liquid. ^{31}P NMR (CDCl_3) δ 31.5.

Example 24

Diacid 33: A solution of benzylether 32 (4.5 g, 18.4 mmol) was dissolved in anhydrous acetonitrile (100mL), chilled to 0°C under a nitrogen atmosphere and treated with TMS bromide (9.73 mL, 74mmol). The reaction mixture was warmed to room temperature and after 15 hours of stirring was concentrated repeatedly with MeOH/water to afford the diacid, which was used directly in the next step. ^{31}P NMR (CDCl_3) δ 31.9.

Example 25

Diphenylphosphonate 34 : Diacid 33 (6.0 g, 27 mmol) was dissolved in toluene and concentrated under reduced pressure three times, dissolved in anhydrous acetonitrile, stirred under a nitrogen atmosphere, and treated with thionyl chloride (20 mL, 270 mmol) by slow addition. The solution was heated to 70°C for two hours, then cooled to room temperature, concentrated and dissolved in anhydrous dichloromethane, chilled to -78°C and treated with phenol (15 g, 162 mmol) and triethylamine (37 mL, 270 mmol). The reaction mixture was warmed to room temperature and stirred for 15 hours, and was then diluted with ice cold dichloromethane, washed with ice cold 1 N. NaOH, washed with ice cold water, dried (MgSO_4), and concentrated under reduced pressure. The resulting residue was used directly in the next step. ^1H NMR (CDCl_3) δ 7.40-7.16 (d, 15H), 4.55 (s, 2H), 3.98-3.84 (m, 2H), 2.55-2.41 (m, 2H); ^{31}P NMR (CDCl_3) δ 22.1.

Example 26

Mono acid 35: Monoacid 35 was prepared using conditions analogous to those used to prepare monoacid 16, except that diphenylphosphonate 34 was substituted for benzylether 15. ^1H NMR (CDCl_3) δ 7.38-7.16 (d, 10H), 4.55 (s, 2H), 3.82-3.60 (m, 3H), 2.33-2.21 (m, 2H); ^{31}P NMR (CDCl_3) δ 29.0.

Example 27

Ethyl lactate phosphonate 36: Ethyl lactate phosphonate 36 was prepared analogously to ethyl lactate phosphonate 18 except monoacid 35 was substituted for monoacid 16. ³¹P NMR (CDCl₃) δ 27.0, 25.6.

Example 28

Ethyl lactate phosphonate with free alcohol 37: Ethyl lactate phosphonate with free alcohol 37 was prepared analogously to ethyl lactate phosphonate with free alcohol 19 except that ethyl lactate phosphonate 36 was substituted for ethyl lactate phosphonate 18. ³¹P NMR (CDCl₃) δ 28.9, 26.8.

Example 29

Triflate 38: A solution of the alcohol 37 (663 mg, 2.19 mmol) and 2,6-lutidine (0.385 mL, 3.31 mmol, Aldrich) in CH₂Cl₂ (5 mL) was stirred at -45°C as trifluoromethanesulfonic anhydride (0.48 mL, 2.85 mmol, Aldrich) was added. The solution was stirred for 1.5 h at -45°C, diluted with ice-cold water (50 mL), and extracted with EtOAc (30 mL x 2). The combined extracts were washed with ice cold water (50 mL), dried (MgSO₄), and concentrated under reduced pressure to obtain a crude mixture of two diastereomers (910 mg, 96%, 1:3 ratio): ¹H NMR (acetone-d₆) δ 7.48-7.37 (m, 2H), 7.37-7.18 (m, 3H), 5.2-4.95 (m, 3H), 4.3-4.02 (m, 2H), 3.38-3.0 (m, 1H), 3.0-2.7 (m, 2H), 2.1-1.9 (m, 1H), 1.52 (d, 1H), 1.4 (d, 2H), 1.4-1.1 (m, 3H); ³¹P NMR (acetone-d₆) δ 21.8 (0.75P), 20.5 (0.25P).

Example 30

The prodrug 39: A solution of the crude triflate 38 (499 mg, 1.15 mmol) and the pyridine 9 (494 mg, 0.877 mmol) in acetone (5 mL) was stirred at room temperature for 16.5 h. The solution was concentrated under reduced pressure to obtain the crude pyridinium salt. To a solution of the crude pyridinium salt in ethanol (10 mL) was added 5 drops of a solution of acetic acid (1 mL) in ethanol (5 mL). The solution was stirred at 0°C as NaBH₄ (~10 mg, Aldrich) was added. The solution was maintained to be pH 3-4 by adding the acetic acid solution. More NaBH₄ and the acetic acid were added until the reduction was completed. After 5.5 h, the mixture was concentrated under reduced pressure and the remaining residue was dissolved in ice-cold saturated NaHCO₃ (50 mL). The product was extracted with ice-

cold EtOAc (30 mL x 2) and the combined extracts were washed with 50% saturated NaHCO₃ (50 mL), dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by a chromatography on silica gel followed by a chromatography on C18 reverse phase column material. Lyophilization of the collected fraction resulted the product

5 39 mixture (376 mg, 50%, ~2.5:1 ratio) as trifluoroacetic acid salt: ¹H NMR (CD₃CN+TFA) δ 7.78 (d, 2H, *J* = 8.7 Hz), 7.52-7.42 (m, 2H); 7.37-7.22 (m 3H), 7.10 (d, 2H, *J* = 8.7 Hz), 5.78 (d, 1H, *J* = 9.0 Hz), 5.64 (m, 1H), 5.50 (br, 1H), 5.08 (m, 2H), 4.31-4.12 (m, 2H), 4.04-3.42 (m, 11H), 3.90 (s, 3H), 3.29 (m, 2H), 3.23 -3.16 (m, 1H), 3.08-2.78 (m, 6H), 2.76-2.27 (m, 5H), 2.23-2.11 (m, 1H), 2.08-1.77 (m, 3H), 1.58 (d, 0.9H, *J* = 7.2 Hz), 1.45 (d, 2.1H, *J* = 6.6 Hz), 1.32-1.20 (m, 3H), 0.95 - 0.84 (m, 6H); ³¹P NMR (CD₃CN+TFA) δ 24.1 and 23.8, 22.2 and 22.1; MS (ESI) 852 (M+H).

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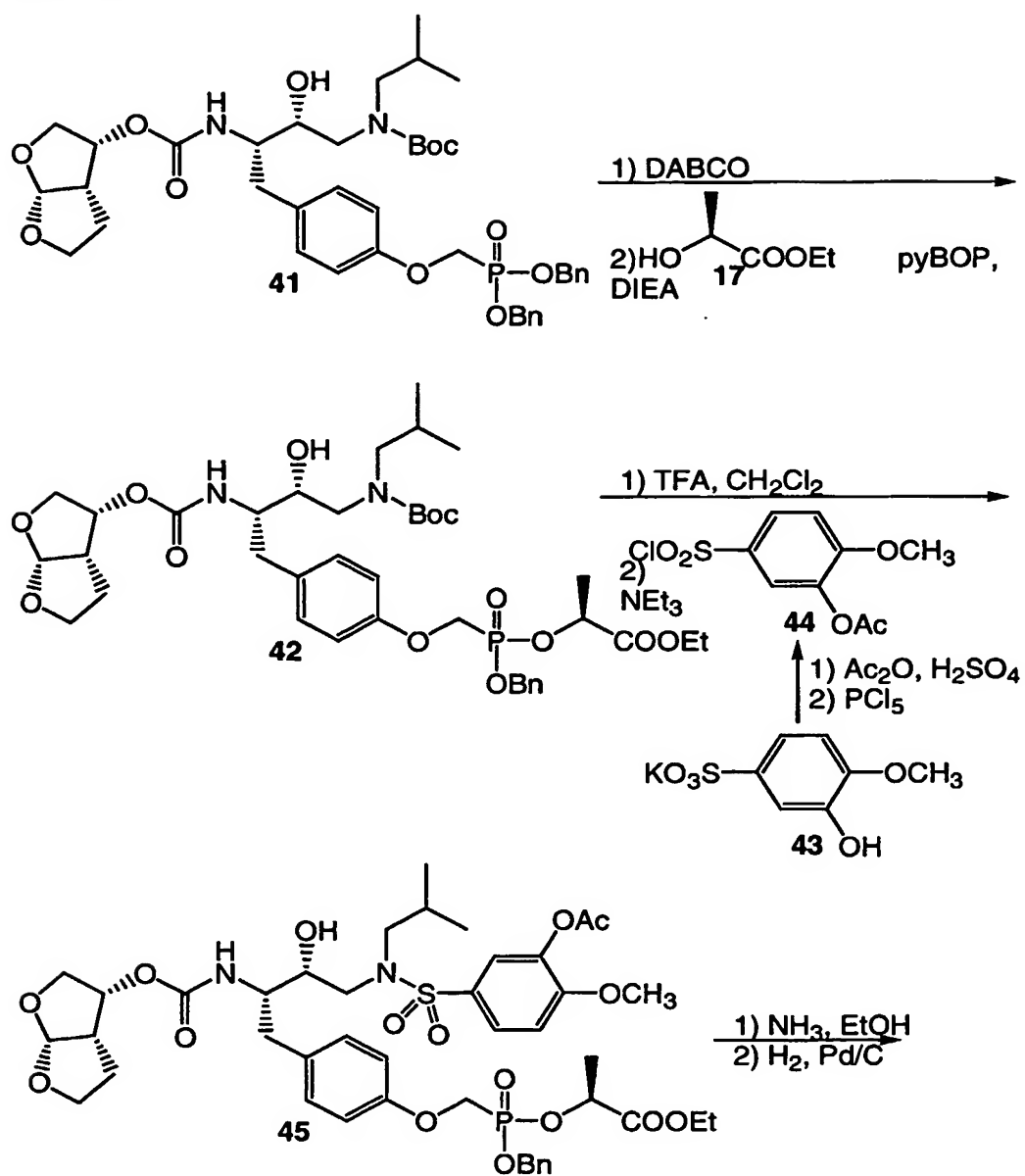
Example 31

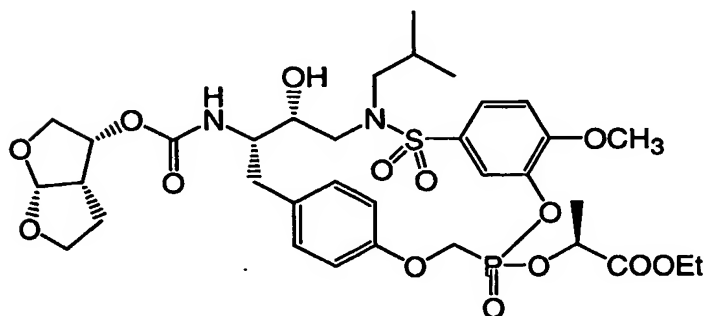
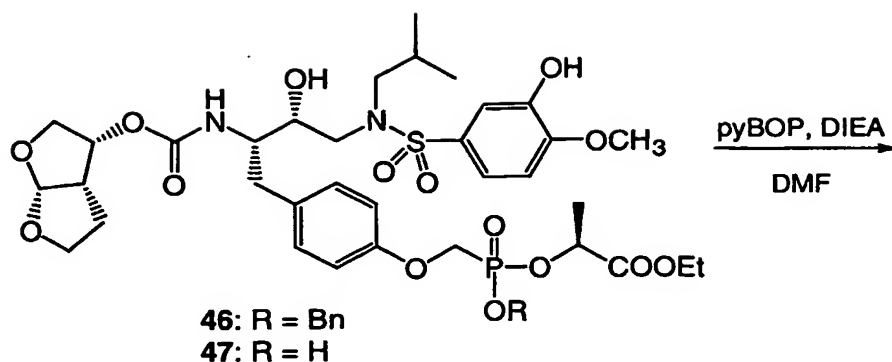
Metabolite 40: To a solution of the prodrug 39 (35.4 mg, 0.037 mmol) in DMSO (0.35 mL) and acetonitrile (0.70 mL) was added 0.1 M PBS buffer (10.5 mL) mixed thoroughly to result

15 a suspension. To the suspension was added porcine liver esterase suspension (0.175 mL, EC3.1.1.1, Sigma). After the suspension was stored in 37°C for 6.5 h, the mixture was filtered through 0.45 um membrane filter and the filtrate was purified by HPLC. The collected fraction was lyophilized to result the product 40 as trifluoroacetic acid salt (28.8

20 mg, 90%): ¹H NMR (D₂O) δ 7.96 (d, 2H, *J* = 8.7 Hz), 7.32 (d, 2H, *J* = 8.7 Hz), 5.89 (d, 1H, *J* = 5.1 Hz), 5.66 (br, 1H), 5.27 (m, 1H), 4.97 (m, 1H), 4.23-4.12 (m, 2H), 4.08 (s, 3H), 4.06-3.10 (m, 14H), 3.03 (dd, 1H, *J* = 14.1 and 6.6 Hz), 2.78-1.97 (m, 9H), 1.66 (d, 3H, *J* = 6.9 Hz), 1.03 (d, 3H, *J* = 7.5 Hz), 1.01 (d, 3H, *J* = 6.9 Hz); ³¹P NMR (CD₃CN+TFA) δ 20.0, 19.8; MS (ESI) 748 (M+H).

Scheme 8





48A: a minor diastereomer (**GS277932**)

48B: a major diastereomer (**GS277933**)

Example 32

Compound 42: The dibenzyl phosphonate **41** (947 mg, 1.21 mmol) was treated with DABCO (140.9 mg, 1.26 mmol, Aldrich) in 4.5 mL toluene to obtain the monoacid (890 mg, 106%). The crude monoacid (890 mg) was dried by evaporation with toluene twice and dissolved in DMF (5.3 mL) with ethyl (*S*)-lactate (0.3 mL, 2.65 mmol, Aldrich) and pyBOP (945 mg, 1.82 mmol, Aldrich) at room temperature. After diisopropylethylamine (0.85 mL, 4.88 mmol, Aldrich) was added, the solution was stirred at room temperature for 4 h and concentrated under reduced pressure to a half volume. The resulting solution was diluted with 5% aqueous HCl (30 mL) and the product was extracted with EtOAc (30 mL x 3). After the combined extracts were dried (MgSO₄) and concentrated, the residue was chromatographed on silica gel to afford the compound **42** (686 mg, 72%) as a mixture of two diastereomers (2:3 ratio): ¹H NMR (CDCl₃) δ 7.46-7.32 (m, 5H), 7.13 (d, 2H, *J* = 8.1 Hz), 6.85 (t, 2H, *J* = 8.1 Hz), 5.65 (m, 1H), 5.35-4.98 (m, 4H), 4.39 (d, 0.8H, *J* = 10.2 Hz), 4.30-4.14 (m, 3.2H), 3.98 (dd, 1H, *J* = 9.3 and 6.0 Hz), 3.92-3.78 (m, 3H), 3.78-3.55 (m, 3H), 3.16-2.68 (m, 6H), 1.85 (m, 1H), 1.74-1.55 (m, 2H), 1.56 (d, 1.8H, *J* = 7.2 Hz), 1.49 (d, 1.2H), 1.48 (s, 9H), 1.30-1.23 (m, 3H), 0.88 (d, 3H, *J* = 6.3 Hz), 0.87 (d, 3H, *J* = 6.3 Hz); ³¹P NMR (CDCl₃) δ 20.8 (0.4P), 19.5 (0.6P); MS (ESI) 793 (M+H).

Example 33

Compound 45: A solution of compound 42 (101 mg, 0.127 mmol) and trifluoroacetic acid (0.27 mL, 3.5 mmol, Aldrich) in CH₂Cl₂ (0.6 mL) was stirred at 0°C for 3.5 h and concentrated under reduced pressure. The resulting residue was dried in vacuum to result the crude amine as TFA salt.

A solution of the crude amine salt and triethylamine (0.072 mL, 0.52 mmol, Aldrich) in CH₂Cl₂ (1 mL) was stirred at 0°C as the sulfonyl chloride 42 (37 mg, 0.14 mmol) was added. After the solution was stirred at 0°C for 4 h and 0.5 h at room temperature, the reaction mixture was diluted with saturated NaHCO₃ (20 mL) and extracted with EtOAc (20 mL x 1; 15 mL x 2). The combined organic fractions were washed with saturated NaCl solution, dried (MgSO₄), and concentrated under reduced pressure. Purification by chromatography on silica gel provided the sulfonamide 45 (85 mg, 72%) as a mixture of two diastereomers (~1:2 ratio): ¹H NMR (CDCl₃) δ 7.45-7.31 (m, 7H), 7.19 (d, 1H, *J* = 8.4 Hz), 7.12 (d, 2H, *J* = 7.8 Hz), 6.85 (m, 2H), 5.65 (d, 1H, *J* = 5.4 Hz), 5.34-5.16 (m, 2H), 5.13-4.97 (m, 2H), 4.97-4.86 (m, 1H), 4.38 (d, 0.7H, *J* = 10.8 Hz), 4.29-4.12 (m, 3.3H), 3.96 (dd, 1H, *J* = 9.3 and 6.3 Hz), 3.89 (s, 3H), 3.92-3.76 (m, 3H), 3.76-3.64 (m, 2H), 3.64-3.56 (br, 1H), 3.34-3.13 (m, 1H), 3.11-2.70 (m, 6H), 2.34 (s, 3H), 1.86 (m, 1H, *J* = 7.0 Hz), 1.75-1.58 (m, 2H), 1.56 (d, 2H, *J* = 7.2 Hz), 1.49 (d, 1H, *J* = 7.2 Hz), 1.29-1.22 (m, 3H), 0.94 (d, 3H, *J* = 6.6 Hz), 0.90 (d, 3H, *J* = 6.9 Hz); ³¹P NMR (CDCl₃) δ 20.7 (0.3P), 19.5 (0.7P); MS (ESI) 921 (M+H).

Example 34

Compound 46: Compound 45 (257 mg, 0.279 mmol) was stirred in a saturated solution of ammonia in ethanol (5 mL) at 0°C for 15 min and the solution was concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided compound 46 (2.6 mg, 84%): ¹H NMR (CDCl₃) δ 7.48-7.34 (m, 4H), 7.22-7.05 (m, 5H), 7.01 (d, 1H, *J* = 8.1 Hz), 6.87-6.80 (m, 2H), 5.68 (d, 1H, *J* = 4.8 Hz), 5.32 (dd, 1.3H, *J* = 8.7 and 1.8 Hz), 5.22 (d, 0.7H, *J* = 9.0 Hz), 5.11-5.00 (m, 3H), 4.47-4.14 (m, 4H), 4.00 (dd, 1H, *J* = 9.9 and 6.6 Hz), 3.93 (s, 3H), 3.95-3.63 (m, 5H), 3.07-2.90 (m, 4H), 2.85-2.75 (m, 1H), 2.75-2.63 (m, 2H), 1.88-1.67 (m, 3H), 1.65-1.55 (m, 2H), 1.57 (d, 2H, *J* = 6.9 Hz), 1.50 (d, 1H, *J* = 7.2 Hz), 1.31-1.20 (m, 3H), 0.95 (d, 3H, *J* = 6.6 Hz), 0.88 (d, 3H, *J* = 6.3 Hz); ³¹P NMR (CDCl₃) δ 20.7 (0.3P), 19.6 (0.7P); MS (ESI) 879 (M+H).

Example 35

Compound 47: A mixture of compound 46 (176 mg, 0.200 mmol) and 10% Pd/C (9.8 mg, Aldrich) in EtOAc (4 mL) and ethanol (1 mL) was stirred under H₂ atmosphere for 3 h at room temperature. After the mixture was filtered through celite, the filtrate was concentrated to dryness to afford compound 47 (158 mg, 100%) as white powder: ¹H NMR (CDCl₃) δ 7.30-7.16 (m, 2H), 7.12 (d, 2H, *J* = 7.5 Hz), 7.01 (d, 1H, *J* = 7.8 Hz), 6.84 (d, 2H, *J* = 7.5 Hz), 5.66 (d, 1H, *J* = 4.5 Hz), 5.13-4.97 (m, 2H), 4.38-4.10 (m, 4H), 3.93 (s, 3H), 4.02-3.66 (m, 6H), 3.13-2.69 (m, 7H), 1.96-1.50 (m, 3H), 1.57 (d, 3H, *J* = 6.6 Hz), 1.26 (t, 3H, *J* = 7.2 Hz), 0.93 (d, 3H, *J* = 6.0 Hz), 0.88 (d, 3H, *J* = 6.0 Hz); ³¹P NMR (CDCl₃) δ 20.1; MS (ESI) 789 (M+H).

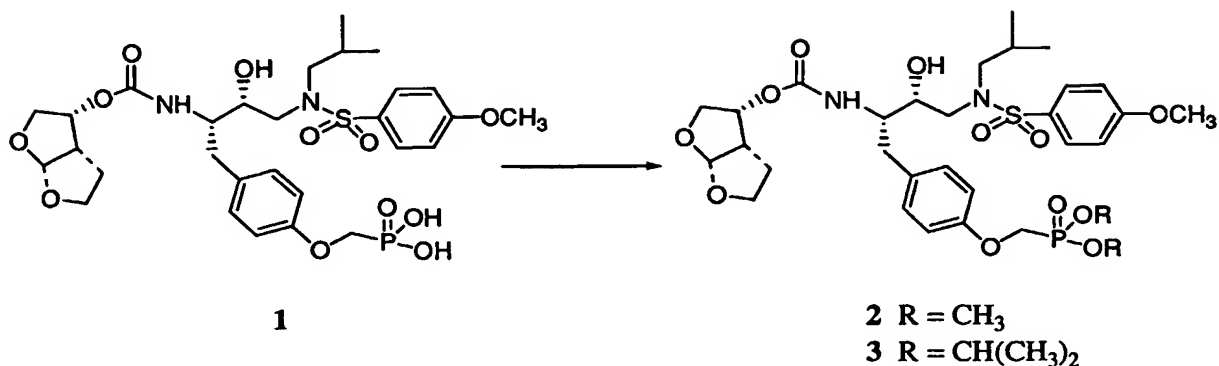
Example 36

Compound 48A and 48B: A solution of pyBOP (191 mg, 0.368 mmol, Aldrich) and diisopropylethylamine (0.1 mL, 0.574 mmol, Aldrich) in DMF (35 mL) was stirred at room temperature as a solution of compound 47 (29 mg, 0.036 mmol) in DMF (5.5 mL) was added over 16 h. After addition, the solution was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was dissolved in ice-cold water and extracted with EtOAc (20 mL x 1; 10 mL x 2). The combined extracts were dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel followed by preparative TLC gave two isomers of structure 48 (1.0 mg, 3.6% and 3.6 mg, 13%). Isomer 48A: ¹H NMR (CDCl₃) δ 7.39 (m, 1H), 7.12 (br, 1H), 7.01 (d, 2H, *J* = 8.1 Hz), 6.98 (br, 1H), 6.60 (d, 2H, *J* = 8.1 Hz), 5.75 (d, 1H, *J* = 5.1 Hz), 5.37-5.28 (m, 2H), 5.18 (q, 1H, *J* = 8.7 Hz), 4.71 (dd, 1H, *J* = 14.1 and 7.5 Hz), 4.29 (m, 3H), 4.15-4.06 (m, 1H), 3.99 (s, 3H), 4.05-3.6 (m, 5H), 3.35 (m, 1H), 3.09 (br, 1H), 2.90-2.78 (m, 3H), 2.2-2.0 (m, 3H), 1.71 (d, 3H, *J* = 6.6 Hz), 1.34 (t, 3H, *J* = 6.9 Hz), 1.01 (d, 3H, *J* = 6.3 Hz), 0.95 (d, 3H, *J* = 6.3 Hz); ³¹P NMR (CDCl₃) δ 17.8; MS (ESI) 793 (M+Na); isomer 48B: ¹H NMR (CDCl₃) δ 7.46 (d, 1H, *J* = 9.3 Hz), 7.24 (br, 1H), 7.00 (d, 2H, *J* = 8.7 Hz), 6.91 (d, 1H, *J* = 8.7 Hz), 6.53 (d, 2H, *J* = 8.7 Hz), 5.74 (d, 1H, *J* = 5.1 Hz), 5.44 (m, 1H), 5.35 (d, 1H, *J* = 9.0 Hz), 5.18 (q, 1H, *J* = 7.2 Hz), 4.68 (dd, 1H, *J* = 14.4 and 6.3 Hz), 4.23 (m, 3H), 4.10 (m, 1H), 4.04 (s, 3H), 3.77-4.04 (m, 6H), 3.46 (dd, 1H, *J* = 12.9 and 11.4 Hz), 3.08 (br, 1H), 2.85 (m, 2H), 2.76 (dd, 1H, *J* = 12.9 and 4.8 Hz), 1.79-2.11 (m, 3H), 1.75 (d, 3H, *J* = 6.6 Hz), 1.70 (m, 2H),

1.27 (t, 3H, $J = 6.9$ Hz), 1.01 (d, 3H, $J = 6.6$ Hz), 0.93 (d, 3H, $J = 6.6$ Hz); ^{31}P NMR (CDCl_3) δ 15.4; MS (ESI) 793 ($\text{M}+\text{Na}$).

Example 1

5



Example 1A

Dimethylphosphonic ester 2 (R = CH₃): To a flask was charged with phosphonic acid 1 (67 mg, 0.1 mmol), methanol (0.1 mL, 2.5 mmol) and 1, 3-dicyclohexylcarbodiimide (83 mg, 0.4 mmol), then pyridine (1 mL) was added under N₂. The resulted mixture was stirred at 60
10 -70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel
15 (isopropanol/CH₂Cl₂, 1% to 7%) to give 2 (39 mg, 56 %) as a white solid. ^1H NMR (CDCl_3) δ 7.71(d, $J = 8.7$ Hz, 2H), 7.15 (d, $J = 8.7\text{Hz}$, 2H), 7.00 (d, $J = 8.7$ Hz, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 5.65 (d, $J = 5.1$ Hz, 1H), 5.10-4.92 (m, 4H), 4.26 (d, $J = 9.9$ Hz, 2H), 3.96 -3.65 (m overlapping s, 15H), 3.14-2.76 (m, 7H), 1.81-1.55 (m, 3H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H); ^{31}P NMR (CDCl_3) δ 21.7; MS (ESI) 723 ($\text{M}+\text{Na}$).

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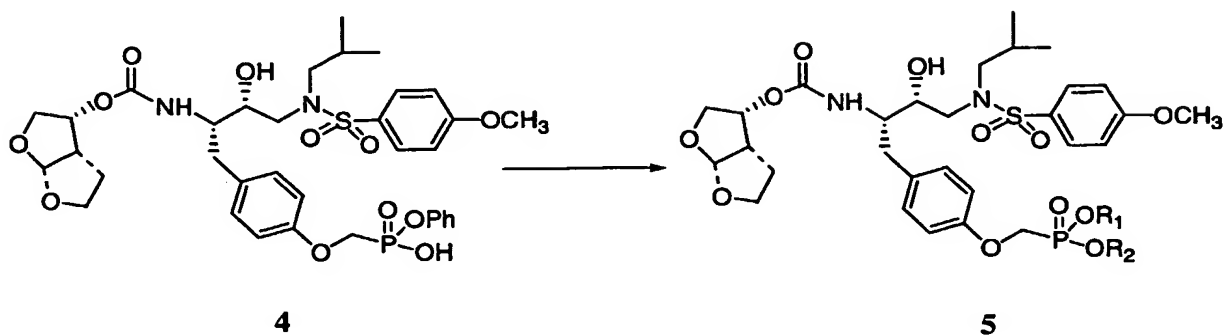
Example 1B

Diisopropylphosphonic ester 3 (R = CH (CH₃)₂) was synthesized in the same manner in 60% yield. ^1H NMR (CDCl_3) δ 7.71(d, $J = 8.7$ Hz, 2H), 7.15 (d, $J = 8.7\text{Hz}$, 2H), 7.15 (d, $J = 8.7$ Hz, 2H), 6.99 (d, $J = 8.7$ Hz, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 5.66 (d, $J = 5.1$ Hz, 1H), 5.08-4.92
25 (m, 3H), 4.16 (d, $J = 10.5$ Hz, 2H), 3.98 -3.68 (m overlapping s, 9H), 3.16-2.78 (m, 7H),

1.82-1.56 (m, 3H), 1.37 (t, $J = 6.3$ Hz, 6H), 0.93 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.3; MS (ESI) 779 ($\text{M}+\text{Na}$).

Example 2

5



| Compound | R ₁ | R ₂ |
|----------|----------------|------------------------|
| 5a | OPh | mix-Hba-Et |
| 5b | OPh | (<i>S</i>)-Hba-Et |
| 5c | OPh | (<i>S</i>)-Hba-tBu |
| 5d | OPh | (<i>S</i>)-Hba-EtMor |
| 5e | OPh | (<i>R</i>)-Hba-Et |

Example 2A

- 10 Monolactate **5a** ($\text{R}_1 = \text{OPh}$, $\text{R}_2 = \text{Hba-Et}$): To a flask was charged with monophenyl phosphonate **4** (250 mg, 0.33 mmol), 2-hydroxy-*n*-butyric acid ethyl ester (145 mg, 1.1 mmol) and 1, 3-dicyclohexylcarbodiimide (226 mg, 1.1 mmol), then pyridine (2.5 mL) was added under N_2 . The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was
- 15 evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH_4Cl , brine and water, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by chromatography on silica gel ($\text{EtOAc}/\text{CH}_2\text{Cl}_2$, 1:1) to give **5a** (150 mg, 52 %) as a white solid. ^1H NMR (CDCl_3) δ 7.70 (d, $J = 8.7$ Hz, 2H), 7.37-7.19 (m, 5H), 7.14 (d, $J = 8.7$ Hz, 2H), 7.00 (d, $J = 8.7$ Hz, 2H), 6.91 (d, $J = 8.7$ Hz, 1H), 6.86 (d, $J = 8.7$
- 20 Hz, 1H), 5.65 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96-3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.21 (m, 3H), 1.04-0.86 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.5 and 15.1; MS (ESI) 885 ($\text{M}+\text{Na}$).

Example 2B

Monolactate **5b** (R1 = OPh, R2 = (*S*)-Hba-Et): To a flask was charged with monophenyl phosphonate **4** (600 mg, 0.8 mmol), (*S*)-2-hydroxy-n-butyric acid ethyl ester (317 mg, 2.4 mmol) and 1, 3-dicyclohexylcarbodiimide (495 mg, 2.4 mmol), then pyridine (6 mL) was added under N₂. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH₂Cl₂, 1:1) to give **5b** (360 mg, 52 %) as a white solid. ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.37-7.19 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96-3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.23 (m, 3H), 1.04-0.86 (m, 6H); ³¹P NMR (CDCl₃) δ 17.5 and 15.2; MS (ESI) 885 (M+Na).

Example 2C

Monolactate **5c** (R1 = OPh, R2 = (*S*)-Hba-tBu): To a flask was charged with monophenyl phosphonate **4** (120 mg, 0.16 mmol), tert-butyl (*S*)-2-hydroxybutyrate (77 mg, 0.48 mmol) and 1, 3-dicyclohexylcarbodiimide (99 mg, 0.48 mmol), then pyridine (1 mL) was added under N₂. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH₂Cl₂, 1:1) to give **5c** (68 mg, 48 %) as a white solid. ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.37-7.19 (m, 5H), 7.14 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.64 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96-3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.44 (d, J = 11 Hz, 9H), 1.04-0.86 (m, 9H); ³¹P NMR (CDCl₃) δ 17.5 and 15.2; MS (ESI) 913 (M+Na).

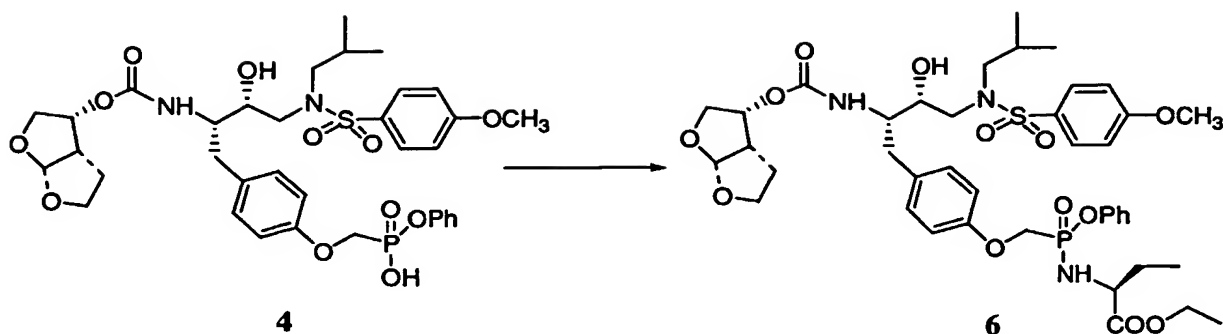
Example 2D

Monolactate **5d** (R1 = OPh, R2 = (*S*)-Lac-EtMor): To a flask was charged with monophenyl phosphonate **4** (188 mg, 0.25 mmol), (*S*)-lactate ethylmorpholine ester (152 mg, 0.75 mmol)

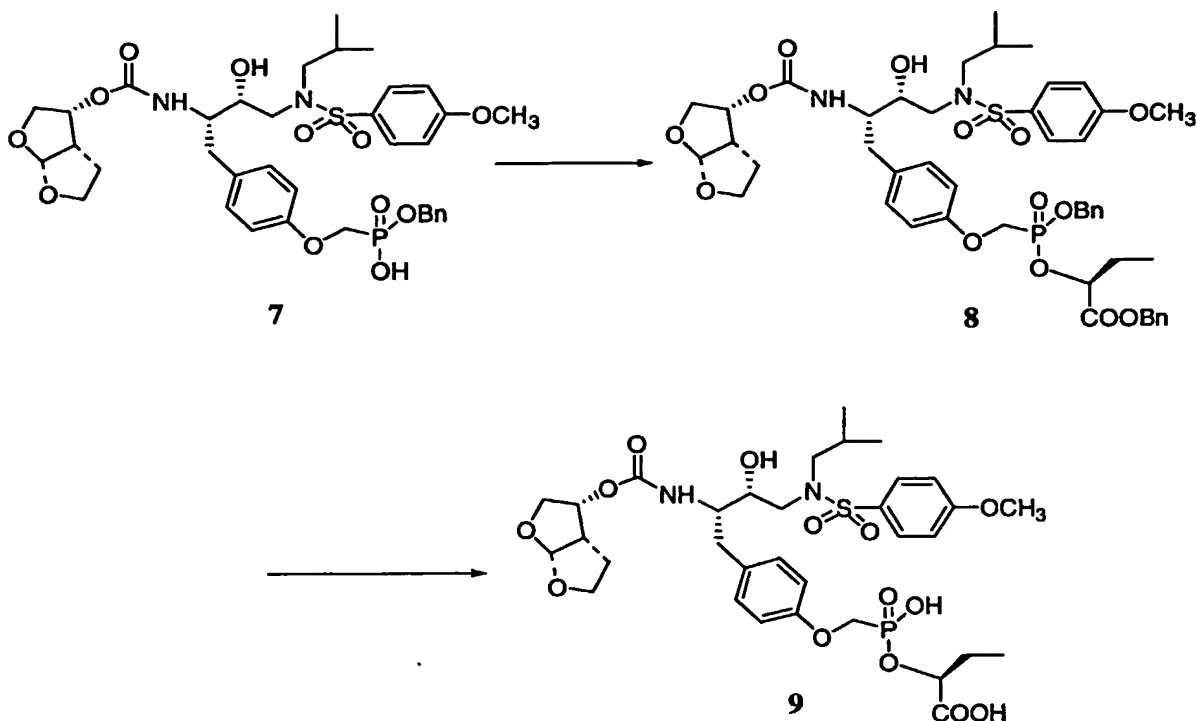
and 1, 3-dicyclohexylcarbodiimide (155 mg, 0.75 mmol), then pyridine (2mL) was added under N₂. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was washed with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol/CH₂Cl₂, 1:9) to give **5d** (98 mg, 42 %) as a white solid. ¹H NMR (CDCl₃) δ 7.72 (d, J = 8.7 Hz, 2H), 7.34–7.20 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.21–4.99 (m, 3H), 4.57–4.20 (m, 4H), 3.97–3.63 (m overlapping s, 13H), 3.01–2.44 (m, 13H), 1.85–1.50 (m, 6H), 0.92 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5, 3H); ³¹P NMR (CDCl₃) δ 17.4 and 15.3; MS (ESI) 934(M).

Example 2E

Monolactate **5e** (R1 = OPh, R2 = (*R*)-Hba-Et): To a flask was charged with monophenyl phosphonate **4** (600 mg, 0.8 mmol), (*R*)-2-hydroxy-*n*-butyric acid ethyl ester (317 mg, 2.4 mmol) and 1, 3-dicyclohexylcarbodiimide (495 mg, 2.4 mmol), then pyridine (6 mL) was added under N₂. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH₂Cl₂, 1:1) to give **5e** (345 mg, 50 %) as a white solid. ¹H NMR (CDCl₃) δ 7.70 (d, J = 8.7 Hz, 2H), 7.37–7.19 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.10–4.95 (m, 3H), 4.57–4.39 (m, 2H), 4.26 (m, 2H), 3.96–3.68 (m overlapping s, 9H), 3.15–2.77 (m, 7H), 1.81–1.55 (m, 5H), 1.23 (m, 3H), 1.04–0.86 (m, 6H); ³¹P NMR (CDCl₃) δ 17.5 and 15.1; MS (ESI) 885 (M+Na).

**Example 3**

Monoamidate **6**: To a flask was charged with monophenyl phosphonate **4** (120 mg, 0.16 mmol), L-alanine butyric acid ethyl ester hydrochloride (160 mg, 0.94 mmol) and 1, 3-dicyclohexylcarbodiimide (132 mg, 0.64 mmol), then pyridine (1 mL) was added under N₂. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol/CH₂Cl₂, 1:9) to give **6** (55 mg, 40 %) as a white solid. ¹H NMR (CDCl₃) δ 7.72 (d, J = 8.7 Hz, 2H), 7.37-7.23 (m, 5H), 7.16 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.90-6.83 (m, 2H), 5.65 (d, J = 5.1 Hz, 1H), 5.10-4.92 (m, 3H), 4.28 (m, 2H), 3.96-3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.23 (m, 3H), 1.04-0.86 (m, 6H); ³¹P NMR (CDCl₃) δ 20.7 and 19.6; MS (ESI) 884(M+Na).

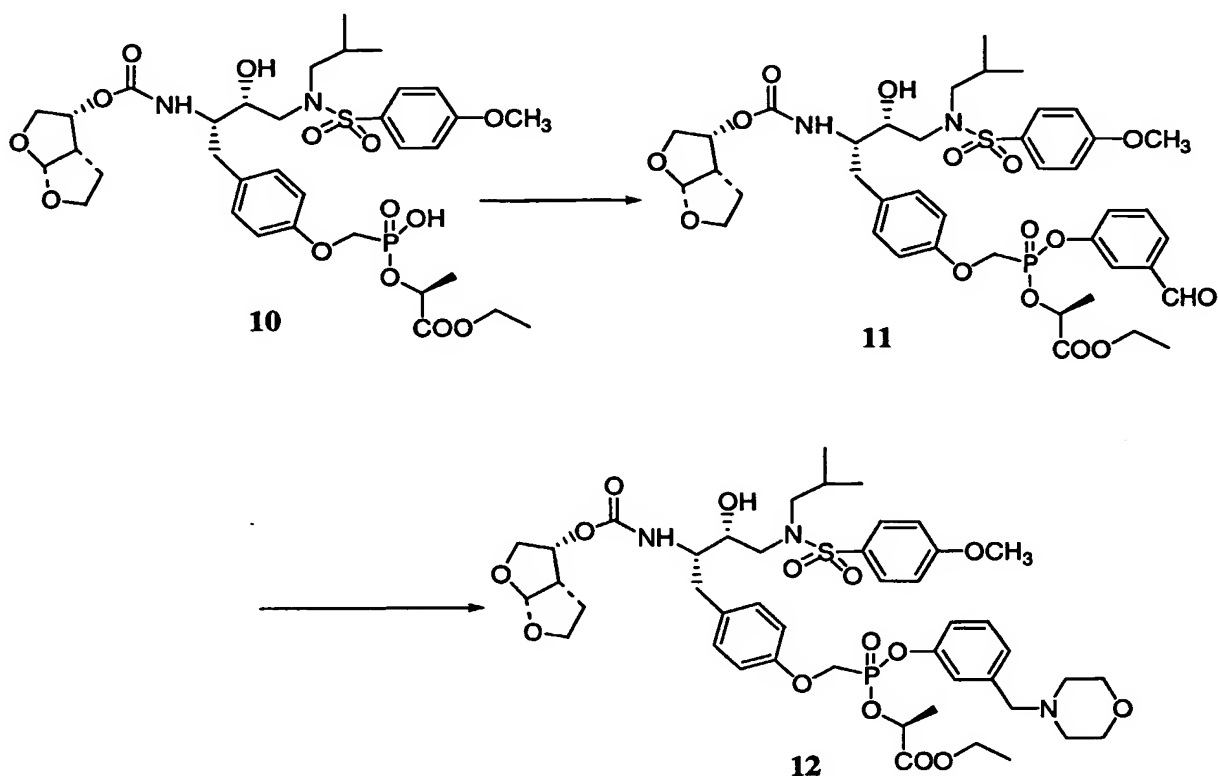
**Example 4A**

Compound 8: To a stirred solution of monobenzyl phosphonate **7** (195 mg, 0.26mmol) in 1 mL of DMF at room temperature under N₂ was added benzyl-(s)-lactate (76 mg, 0.39 mmol) and PyBOP (203 mg, 0.39mmol), followed by DIEA (181μL, 1 mmol). After 3 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) to give **8** (120 mg, 50%) as a white solid. ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.38-7.34 (m, 5H), 7.12 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.81(d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.24-4.92 (m, 7H), 4.28 (m, 2H), 3.96 -3.67 (m overlapping s, 9H), 3.16-2.76 (m, 7H), 1.95-1.62 (m, 5H), 0.99-0.87 (m, 9H); ³¹P NMR (CDCl₃) δ 21.0 and 19.7; MS (ESI) 962 (M+Na).

Example 4B

Compound 9: A solution of compound **8** (100 mg) was dissolved in EtOH/ EtOAc (9 mL/ 3mL), treated with 10 % Pd/C (10 mg) and was stirred under H₂ atmosphere (balloon) for 1.5 h. The catalyst was removed by filtration through celite. The filtered was evaporated under reduced pressure, the residue was triturated with ether and the solid was collected by filtration to afford the compound **9** (76mg, 94%) as a white solid. ¹H NMR (CD₃OD) δ 7.76 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.03-4.95 (m, 2H), 4.28 (m, 2H), 3.90 -3.65 (m overlapping s, 9H).

9H), 3.41 (m, 2H), 3.18-2.78 (m, 5H), 2.44 (m, 1H), 1.96 (m, 3H), 1.61 (m, 2H), 1.18 (m, 3H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.87 (d, $J = 6.3$ Hz, 3H); ^{31}P NMR (CD_3OD) δ 18.3; MS (ESI) 782 ($\text{M}+\text{Na}$).



5

Example 5A

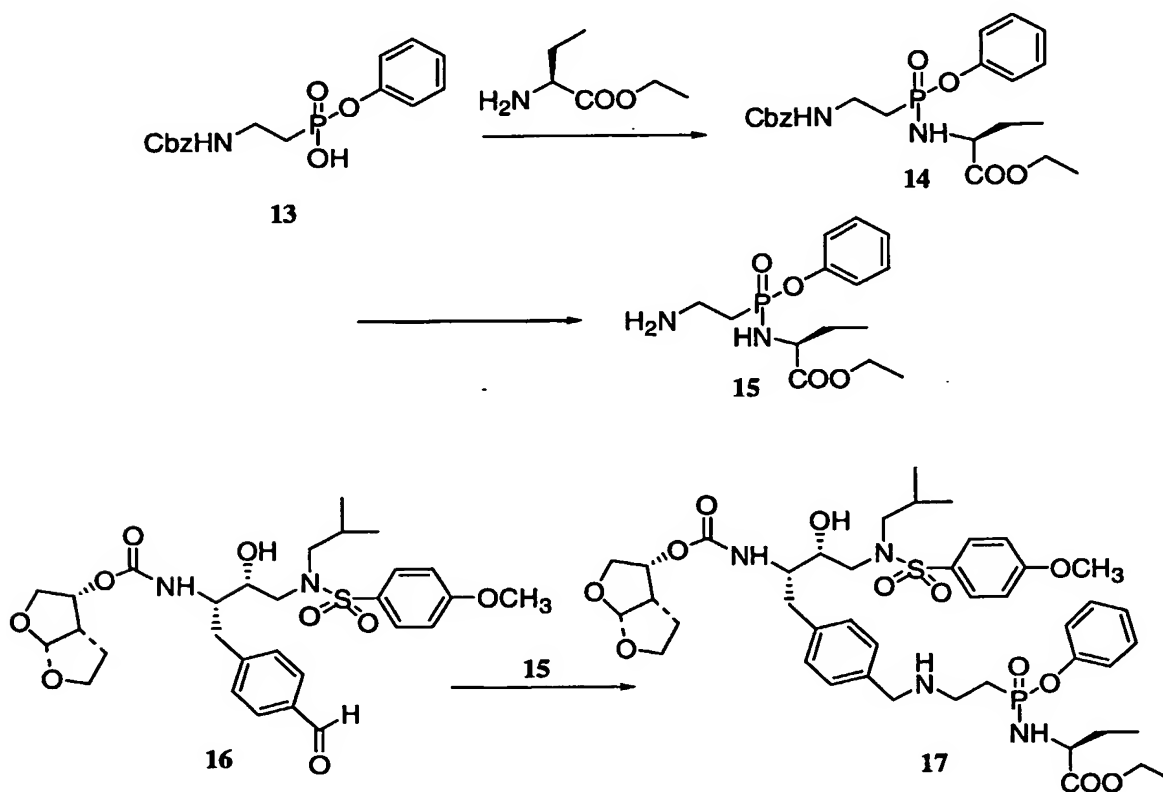
Compound 11: To a stirred solution of compound 10 (1 g, 1.3mmol) in 6 mL of DMF at room temperature under N_2 was added 3-hydroxybenzaldehyde (292 mg, 2.6 mmol) and PyBOP (1 g, 1.95mmol), followed by DIEA (0.9 mL, 5.2 mmol). After 5 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) to give 11 (800 mg, 70%) as a white solid. ^1H NMR (CDCl_3) δ 9.98 (s, 1H), 7.79-6.88 (m, 12H), 5.65 (m, 1H), 5.21-4.99 (m, 3H), 4.62-4.16 (m, 4H), 3.99 -3.61 (m overlapping s, 9H), 3.11-2.79 (m, 5H), 1.85-1.53 (m, 6H), 1.25 (m, 3H), 0.90 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.9 and 15.9; MS (ESI) 899 ($\text{M}+\text{Na}$).

15

Example 5B

Compound 12: To a stirred solution of compound 11 (920 mg, 1.05 mmol) in 10 mL of ethyl acetate at room temperature under N_2 was added morpholine (460 mg, 5.25 mmol) and acetic

acid (0.25 mL, 4.2 mmol), followed by sodium cyanoborohydride (132 mg, 2.1 mmol). After 20h, the solvent was removed under reduced pressure, and the residue was diluted with ethyl acetate and the combined organic phase was washed with NH₄Cl, brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol / CH₂Cl₂, 6%) to give 12 (600 mg, 60%) as a white solid. ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.27 (m, 4H), 7.15 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.89 (m, 2H), 5.65 (m, 1H), 5.21-5.02 (m, 3H), 4.58-4.38 (m, 2H), 4.21-4.16 (m, 2H), 3.99-3.63 (m overlapping s, 15H), 3.47 (s, 2H), 3.18-2.77 (m, 7H), 2.41 (s, 4H), 1.85-1.53 (m, 6H), 1.25 (m, 3H), 0.90 (m, 6H); ³¹P NMR (CDCl₃) δ 17.4 and 15.2; MS (ESI) 971 (M+Na).



Example 6A

Compound 14: To a stirred solution of compound 13 (1 g, 3 mmol) in 30 mL of acetonitrile at room temperature under N₂ was added thionyl chloride (0.67 mL, 9 mmol). The resulted mixture was stirred at 60-70°C for 0.5 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 30 mL of DCM, followed by DIEA (1.7 mL, 10 mmol), L-alanine butyric acid ethyl ester hydrochloride (1.7 g, 10 mmol) and TEA (1.7 mL, 12 mmol). After 4h at room temperature, the solvent was removed under

reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by chromatography on silica gel (Hexane/EtOAc 1:1) to give **14** (670 mg, 50%) as a yellow oil. ^1H NMR (CDCl_3) δ 7.33-7.11 (m, 10H), 5.70 (m, 1H), 5.10 (s, 2H), 4.13-3.53 (m, 5H), 2.20-2.10 (m, 2H), 1.76-1.55 (m, 2H), 1.25-1.19 (m, 3H), 0.85-0.71 (m, 3H); ^{31}P NMR (CDCl_3) δ 30.2 and 29.9; MS (ESI) 471 ($\text{M}+\text{Na}$).

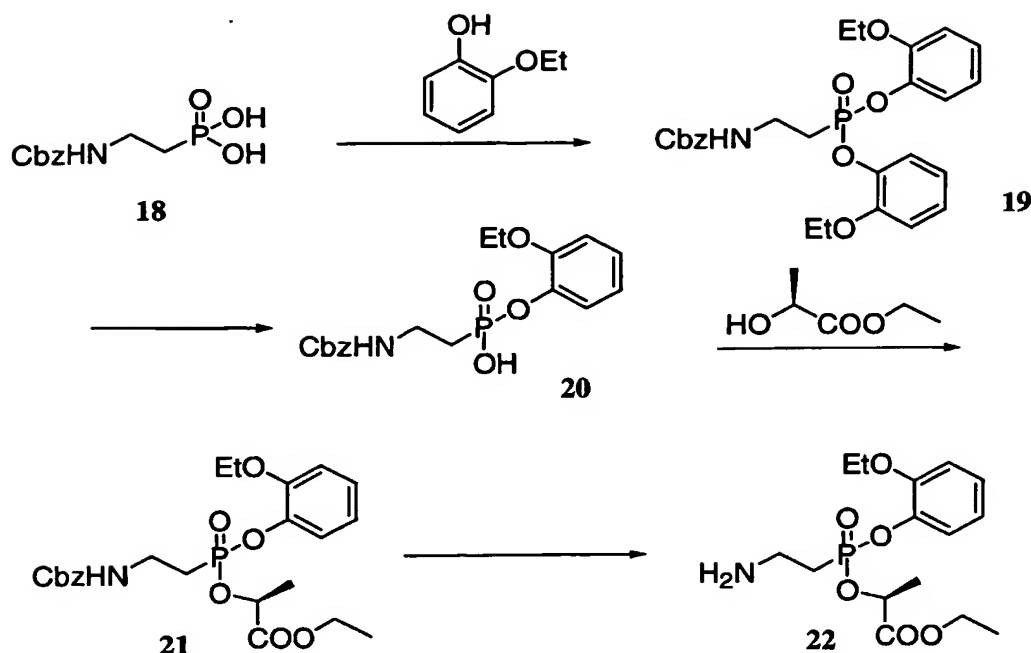
Example 6B

Compound **15**: A solution of compound **14** (450mg) was dissolved in 9 mL of EtOH, then 0.15 mL of acetic acid and 10 % Pd/C (90 mg) was added. The resulted mixture was stirred under H_2 atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **15** (300mg, 95%) as a colorless oil. ^1H NMR (CDCl_3) δ 7.29-7.12 (m, 5H), 4.13-3.53 (m, 5H), 2.20-2.10 (m, 2H), 1.70-1.55 (m, 2H), 1.24-1.19 (m, 3H), 0.84-0.73(m, 3H); ^{31}P NMR (CDCl_3) δ 29.1 and 28.5; MS (ESI) 315 ($\text{M}+1$).

Example 6C

Monoamidate **17**: To a stirred solution of compound **16** (532 mg, 0.9 mmol) in 4 mL of 1,2-dichloroethane was added compound **15** (300 mg, 0.96 mmol) and MgSO_4 (50 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (1.3 mL, 23 mmol) and sodium cyanoborohydride (1.13 g, 18 mmol) were added. The reaction mixture was stirred at room temperature for 1 h under argon. Then aqueous NaHCO_3 (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH / EtOAc, 1/9) to give **17** (600 mg, 60%) as a white solid. ^1H NMR (CDCl_3) δ 7.73 (d, $J = 8.7$ Hz, 2H), 7.33-7.13 (m, 9H), 7.00 (d, $J = 8.7$ Hz, 2H), 5.65 (d, $J = 5.4$ Hz, 1H), 5.11-4.98 (m, 2H), 4.22 -3.68 (m overlapping s, 15H), 3.20-2.75 (m, 9H), 2.21-2.10 (m, 2H), 1.88-1.55(m, 5H), 1.29-1.19 (m, 3H), 0.94-0.70 (m, 9H); ^{31}P NMR (CDCl_3) δ 31.8 and 31.0; MS (ESI) 889 (M).

Example 7

**Example 7A**

Compound **19**: To a stirred solution of compound **18** (3.7 g, 14.3 mmol) in 70 mL of acetonitrile at room temperature under N₂ was added thionyl chloride (6.3 mL, 86 mmol). The resulted mixture was stirred at 60-70°C for 2 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 150 mL of DCM, followed by TEA (12 mL, 86 mmol) and 2-ethoxyphenol (7.2 mL, 57.2 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with ethyl acetate and washed with brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM/EtOAc 9:1) to give **19** (4.2 g, 60%) as a yellow oil. ¹H NMR (CDCl₃) δ 7.32-6.83 (m, 13H), 5.22 (m, 1H), 5.12 (s, 2H), 4.12-3.73 (m, 6H), 2.52-2.42 (m, 2H), 1.41-1.37 (m, 6H); ³¹P NMR (CDCl₃) δ 25.4; MS (ESI) 522 (M+Na).

Example 7B

Compound **20**: A solution of compound **19** (3 g, 6 mmol) was dissolved in 70 mL of acetonitrile at 0°C, then 2N NaOH (12 mL, 24 mmol) was added dropwisely. The reaction mixture was stirred at room temperature for 1.5 h. Then the solvent was removed under reduced pressure, and the residue diluted with water and extracted with ethyl acetate. The aqueous layer was acidified with conc. HCl to PH = 1, then extracted with ethyl acetate,

combined the organic layer and dried over Na_2SO_4 , filtered and concentrated to give compound **20** (2 g, 88%) as a off-white solid. ^1H NMR (CDCl_3) δ 7.33-6.79 (m, 9H), 5.10 (s, 2H), 4.12-3.51 (m, 6H), 2.15-2.05 (m, 2H), 1.47-1.33 (m, 3H); ^{31}P NMR (CDCl_3) δ 30.5; MS (ESI) 380 (M+1).

5

Example 7C

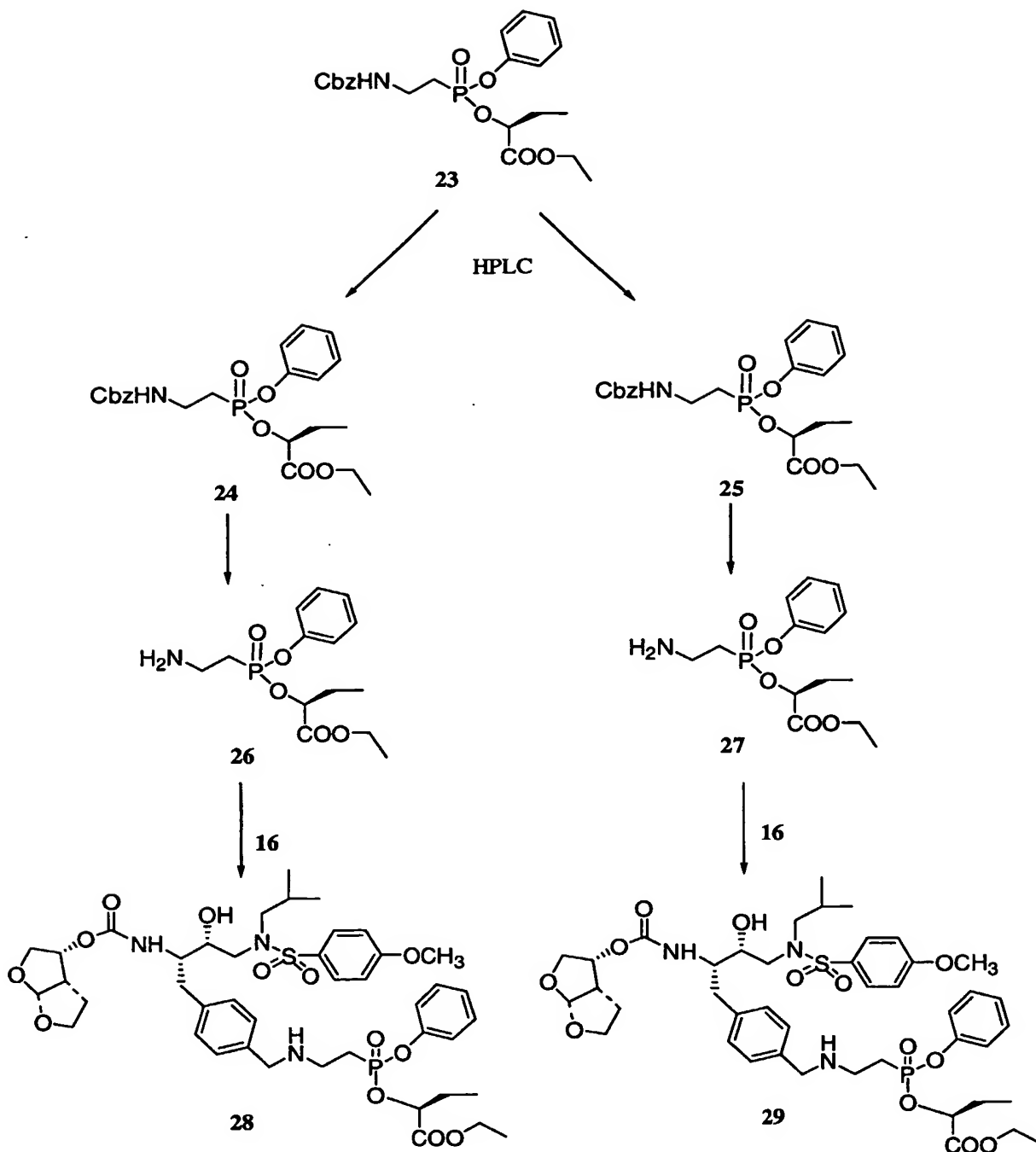
Compound **21**: To a stirred solution of compound **20** (1 g, 2.6 mmol) in 20 mL of acetonitrile at room temperature under N_2 was added thionyl chloride (1.1 mL, 15.6 mmol). The resulted mixture was stirred at 60-70°C for 45 min. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 25 mL of DCM, followed by TEA (1.5 mL, 10.4 mmol) and (S) lactate ethyl ester (0.9 mL, 7.8 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by chromatography on silica gel (DCM / EtOAc 3:1) to give **21** (370 mg, 30%) as a yellow oil. ^1H NMR (CDCl_3) δ 7.33- 6.84 (m, 9H), 6.17-6.01 (m, 1H), 5.70 (m, 1H), 5.18-5.01 (m, 3H), 4.25-4.04 (m, 4H), 3.78-3.57 (m, 2H), 2.38-2.27 (m, 2H), 1.5-1.23 (m, 9H); ^{31}P NMR (CDCl_3) δ 29.2 and 27.3; MS (ESI) 502 (M+Na).

15

Example 7D

Compound **22**: A solution of compound **21** (370mg) was dissolved in 8 mL of EtOH, then 0.12 mL of acetic acid and 10 % Pd/C (72 mg) was added. The resulted mixture was stirred under H_2 atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **22** (320mg, 96%) as a colorless oil. ^1H NMR (CDCl_3) 7.27- 6.86 (m, 4H), 5.98 (s, 2H), 5.18-5.02 (m, 1H), 4.25-4.06 (m, 4H), 3.34-3.24 (m, 2H), 2.44-2.30 (m, 2H), 1.62-1.24 (m, 9H); ^{31}P NMR (CDCl_3) δ 28.3 and 26.8; MS (ESI) 346 (M+1).

25



Example 8A

Compound **24**: Compound **23** was purified using a Dynamax SD-200 HPLC system. The mobile phase consisted of acetonitrile: water (65:35, v/v) at a flow rate of 70 mL/ min. The injection volume was 4 mL. The detection was by fluorescence at 245 nm and peak area ratios were used for quantitations. Retention time was 8.2 min for compound **24** as yellow oil. ¹H NMR (CDCl₃) δ 7.36-7.19 (m, 10H), 5.88 (m, 1H), 5.12 (s, 2H), 4.90-4.86 (m, 1H),

4.26-4.12 (m, 2H), 3.72-3.61(m, 2H), 2.36-2.29 (m, 2H), 1.79-1.74 (m, 2H); 1.27 (t, J = 7.2 Hz, 3H), 0.82 (t, J = 7.2 Hz, 3H); ^{31}P NMR (CDCl_3) δ 28.3; MS (ESI) 472 (M+Na).

Example 8B

- 5 Compound **25** was purified in the same manner and retention time was 7.9 min for compound **25** as yellow oil. ^1H NMR (CDCl_3) δ 7.34-7.14 (m, 10H), 5.75 (m, 1H), 5.10 (s, 2H), 4.96-4.91 (m, 1H), 4.18-4.12 (m, 2H), 3.66-3.55(m, 2H), 2.29-2.19 (m, 2H), 1.97-1.89 (m, 2H); 1.21 (t, J = 7.2 Hz, 3H), 0.97 (t, J = 7.2 Hz, 3H); ^{31}P NMR (CDCl_3) δ 26.2; MS (ESI) 472 (M+Na).

10

Example 8C

- Compound **26**: A solution of compound **24** (1 g) was dissolved in 20 mL of EtOH, then 0.3 mL of acetic acid and 10 % Pd/C (200 mg) was added. The resulted mixture was stirred under H_2 atmosphere (balloon) for 4 h. After filtration through celite, the filtered was
15 evaporated under reduced pressure to afford the compound **26** (830mg, 99 %) as a colorless oil. ^1H NMR (CDCl_3) δ 7.46-7.19 (m, 5H), 4.92-4.81 (m, 1H), 4.24-4.21 (m, 2H), 3.41-3.28 (m, 2H), 2.54-2.38 (m, 2H), 1.79-1.74 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H); ^{31}P NMR (CDCl_3) δ 26.9; MS (ESI) 316 (M+1).

Example 8D

- 20 Compound **27**: A solution of compound **25** (700g) was dissolved in 14 mL of EtOH, then 0.21 mL of acetic acid and 10 % Pd/C (140 mg) was added. The resulted mixture was stirred under H_2 atmosphere (balloon) for 4 h. After filtration through celite, the filtered was
25 evaporated under reduced pressure to afford the compound **27** (510mg, 98 %) as a colorless oil. ^1H NMR (CDCl_3) δ 7.39-7.18 (m, 5H), 4.98-4.85 (m, 1H), 4.25-4.22 (m, 2H), 3.43-3.28 (m, 2H), 2.59-2.41 (m, 2H), 1.99-1.85 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 1.02 (t, J = 7.2 Hz, 3H); ^{31}P NMR (CDCl_3) δ 24.2; MS (ESI) 316 (M+1).

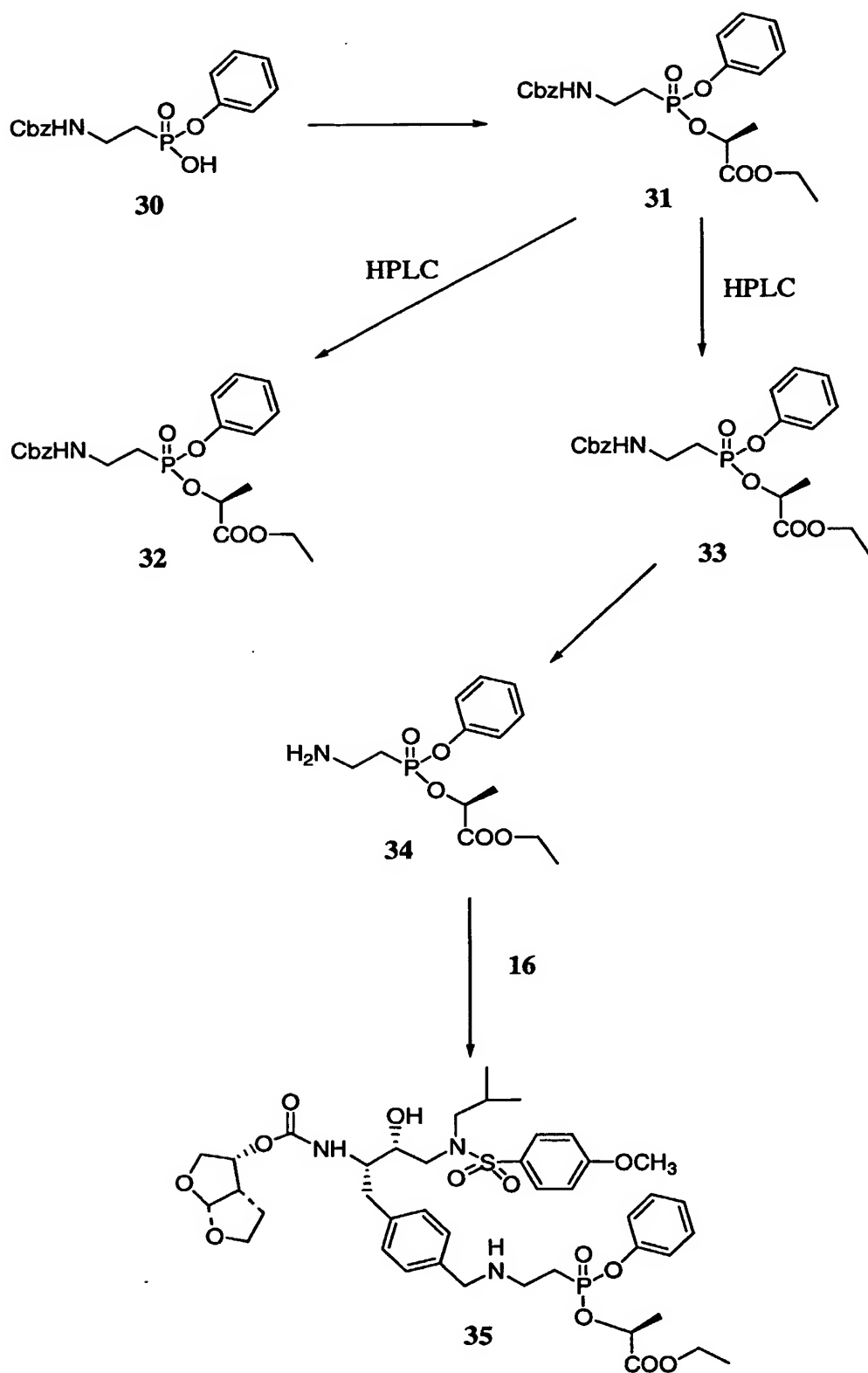
Example 8E

- 30 Compound **28**: To a stirred solution of compound **16** (1.18 g, 2 mmol) in 9 mL of 1,2-dichloroethane was added compound **26** (830 mg, 2.2 mmol) and MgSO_4 (80 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (0.34

mL, 6 mmol) and sodium cyanoborohydride (251mg, 4 mmol) were added. The reaction mixture was stirred at room temperature for 2 h under argon. Then aqueous NaHCO₃ (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **28** (880 mg, 50 %) as a white solid. ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.35-7.16 (m, 9H), 6.99 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.03-4.85 (m, 3H), 4.24 -3.67 (m overlapping s, 15H), 3.14-2.70 (m, 9H), 2.39-2.28 (m, 2H), 1.85-1.51 (m, 5H), 1.29-1.25 (m, 3H), 0.93-0.78 (m, 9H); ³¹P NMR (CDCl₃) δ 29.2; MS (ESI) 912 (M+Na).

Example 8F

Compound **29**: To a stirred solution of compound **16** (857 g, 1.45 mmol) in 7 mL of 1,2-dichloroethane was added compound **27** (600 mg, 1.6 mmol) and MgSO₄ (60 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (0.23 mL, 3 mmol) and sodium cyanoborohydride (183mg, 2.9 mmol) were added. The reaction mixture was stirred at room temperature for 2 h under argon. Then aqueous NaHCO₃ (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **29** (650 mg, 50 %) as a white solid. ¹H NMR (CDCl₃) δ 7.72 (d, J = 8.7 Hz, 2H), 7.35-7.16 (m, 9H), 7.00 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.03-4.90 (m, 3H), 4.17 -3.67 (m overlapping s, 15H), 3.16-2.77 (m, 9H), 2.26-2.19 (m, 2H), 1.94-1.53 (m, 5H), 1.26-1.18 (m, 3H), 1.00-0.87 (m, 9H); ³¹P NMR (CDCl₃) δ 27.4; MS (ESI) 912 (M+Na).



Example 9A

Compound **31**: To a stirred solution of compound **30** (20 g, 60 mmol) in 320 mL of toluene at room temperature under N₂ was added thionyl chloride (17.5 mL, 240 mmol) and a few drops of DMF. The resulted mixture was stirred at 60-70°C for 3 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 280 mL of DCM, followed by TEA (50 mL, 360 mmol) and (S) lactate ethyl ester (17 mL, 150 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM / EtOAc, 1:1) to give **31** (24 g, 92 %) as a yellow oil. ¹H NMR (CDCl₃) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); ³¹P NMR (CDCl₃) δ 28.2 and 26.2; MS (ESI) 458 (M+Na).

15 Example 9B

Compound **32**: Compound **31** was purified using a Dynamax SD-200 HPLC system. The mobile phase consisted of acetonitrile: water (60:40, v/v) at a flow rate of 70 mL/ min. The injection volume was 3 mL. The detection was by fluorescence at 245 nm and peak area ratios were used for quantitations. Retention time was 8.1 min for compound **32** as yellow oil. ¹H NMR (CDCl₃) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); ³¹P NMR (CDCl₃) δ 28.2; MS (ESI) 458 (M+Na).

Example 9C

25 Compound **33** was purified in the same manner and retention time was 7.9 min for compound **33** as yellow oil. ¹H NMR (CDCl₃) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); ³¹P NMR (CDCl₃) δ 26.2; MS (ESI) 458 (M+Na).

30 Example 9D

Compound **34**: A solution of compound **33** (3.2 g) was dissolved in 60 mL of EtOH, then 0.9 mL of acetic acid and 10 % Pd/C (640 mg) was added. The resulted mixture was stirred

under H₂ atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **34** (2.7 g, 99 %) as a colorless oil. ¹H NMR (CDCl₃) δ 7.42-7.18 (m, 5H), 6.10 (s, 1H), 5.15-5.02 (m, 1H), 4.24-4.05 (m, 2H), 3.25-3.16 (m, 2H), 2.36-2.21 (m, 2H), 1.61-1.58 (m, 3H), 1.35- 1.18, m, 3H); ³¹P NMR (CDCl₃) δ 26.1; MS (ESI) 302 (M+1).

Example 9E

Compound **35**: To a stirred solution of compound **16** (8.9 g, 15 mmol) in 70 mL of 1,2-dichloroethane was added compound **34** (8.3 g, 23 mmol) and MgSO₄ (80 mg), the resulted mixture was stirred at room temperature under argon for 2.5h, then acetic acid (3 mL, 52.5 mmol) and sodium cyanoborohydride (1.9g, 30 mmol) were added. The reaction mixture was stirred at room temperature for 1.5 h under argon. Then aqueous NaHCO₃ (100 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **35** (8.4 g, 64 %) as a white solid. ¹H NMR (CDCl₃) δ 7.73 (d, J = 8.7 Hz, 2H), 7.36-7.17(m, 9H), 7.00 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.07-4.97 (m, 3H), 4.19 -3.67 (m overlapping s, 13H), 3.15-2.78 (m, 9H), 2.25-2.19 (m, 2H), 1.91-1.54 (m, 6H), 1.24-1.20 (m, 3H), 0.94-0.87 (m, 6H); ³¹P NMR (CDCl₃) δ 27.4; MS (ESI) 876 (M+1).

Resolution of Compound 35 Diastereomers

Analysis was performed on an analytical Daicel Chiralcel OD column, conditions described below, with a total of about 3.5 mg compound **35** free base injected onto the column. This lot was about a 3:1 mixture of major to minor diastereomers where the lactate ester carbon is a 3:1 mix of R and S configurations.

Two injections of 3.8 and 3.5 mg each were made using the conditions described below. The isolated major diastereomer fractions were evaporated to dryness on a rotary evaporator under house vacuum. The chromatographic solvents were displaced by two portions of ethyl acetate followed by a single portion of ethyl acetate – trifluoroacetic acid (about 95:5) and a final high vacuum strip to aid in removal of trace solvents. This yielded the major diastereomer trifluoroacetate salt as a gummy solid.

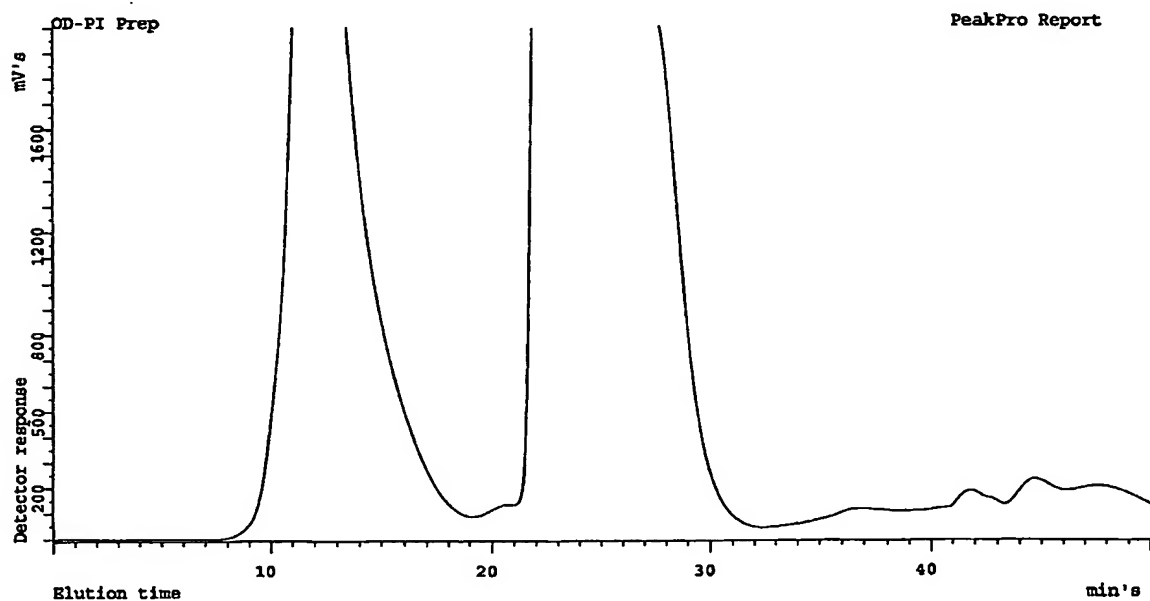
The resolved minor diastereomer was isolated for biological evaluation by an 11 mg injection, performed on an analytical Daicel Chiralcel OD column, using the conditions described in below. The minor diastereomer of 35 was isolated as the trifluoroacetate salt by the conditions described above.

5

Larger scale injections (~ 300 mg 35 per injection) were later performed on a Daicel Chiralcel OD column semi-preparative column with a guard column, conditions described below. A minimal quantity of isopropyl alcohol was added to heptane to dissolve the 3:1 diastereomeric mix of 35 and the resolved diastereomers sample, and the isolated fractions were refrigerated until the eluted mobile phase was stripped.

10

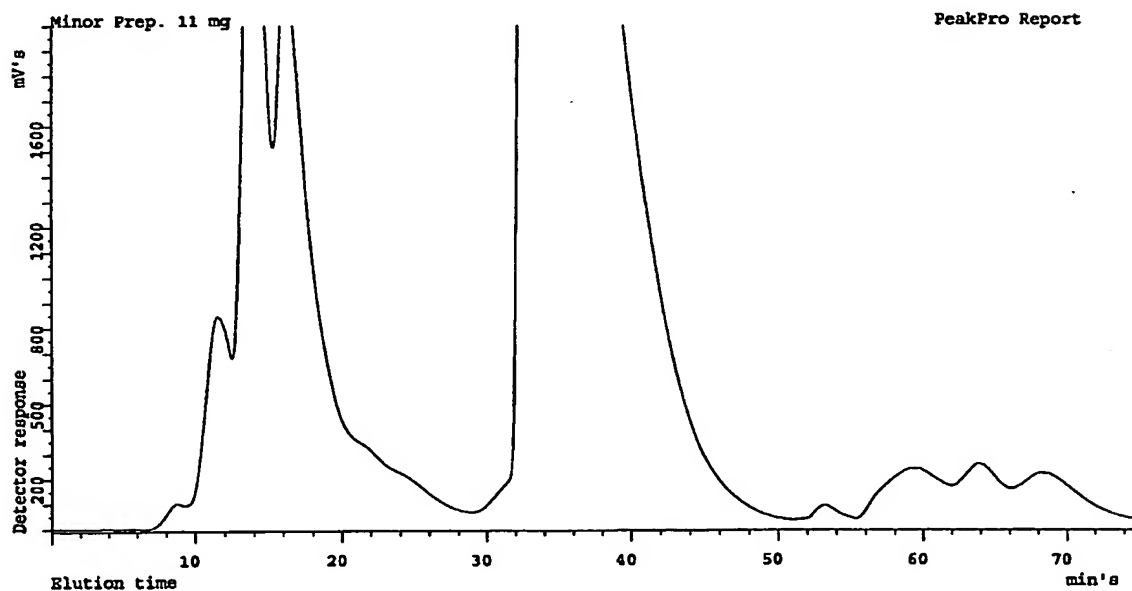
Analytical Column, ~ 4 mg Injection, Heptane – EtOH (20:80) Initial



15

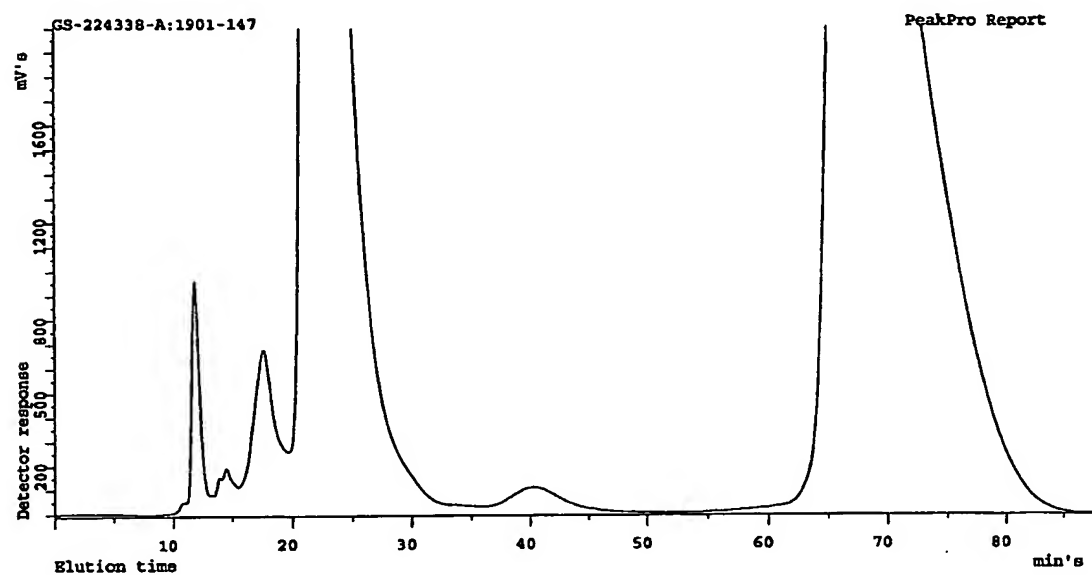
HPLC CONDITIONS

Column : Chiralcel OD, 10 μ m, 4.6 x 250 mm
Mobile Phase : Heptane – Ethyl Alcohol (20:80 initial)
: 100% Ethyl Alcohol (final)
Note: Final began after first peak eluted
Flow Rate : 1.0 mL/min
Run Time : As needed
Detection : UV at 250 nm
Temperature : Ambient
Injection : ~ 4 mg on Column
Sample Prep. : Dissolved in ~ 1 mL heptane –
ethyl alcohol (50:50)
Retention Times : 35 Minor ~ 14 min
: 35 Major ~ 25 min

Analytical Column, ~ 6 mg Injection, Heptane – EtOH (65:35) Initial

HPLC CONDITIONS

| | |
|-----------------|---|
| Column | : Chiralcel OD, 10 μ m, 4.6 x 250 mm |
| Mobile Phase | : Heptane – Ethyl Alcohol (65:35 initial) : Heptane – Ethyl Alcohol (57.5:42.5 intermediate) Note: Intermediate began after impurity peaks eluted : Heptane – Ethyl Alcohol (20:80 final) Note: Final mobile phase began after minor diastereomer eluted |
| Flow Rate | : 1.0 mL/min |
| Run Time | : As needed |
| Detection | : UV at 250 nm |
| Temperature | : Ambient |
| Injection | : ~ 4 mg on Column |
| Sample Prep. | : Dissolved in ~ 1 mL heptane – ethyl alcohol (50:50) |
| Retention Times | : 35 Minor ~ 14 min : 35 Major ~ 40 min |

Semi-Preparative Column, ~ 300 mg Injection, Heptane – EtOH (65:35) Initial

HPLC CONDITIONS

Columns : Chiralcel OD, 20 μ m, 21 x 50 mm (guard)
: Chiralcel OD, 20 μ m, 21 x 250 mm

Mobile Phase : Heptane – Ethyl Alcohol (65:35 initial)
: Heptane – Ethyl Alcohol (50:50 intermediate)
Note: Intermediate began after minor
diastereomer peak eluted
: Heptane – Ethyl Alcohol (20:80 final)
Note: Final mobile phase began after major
diastereomer began to elute

Flow Rate : 10.0 mL/min

Run Time : As needed

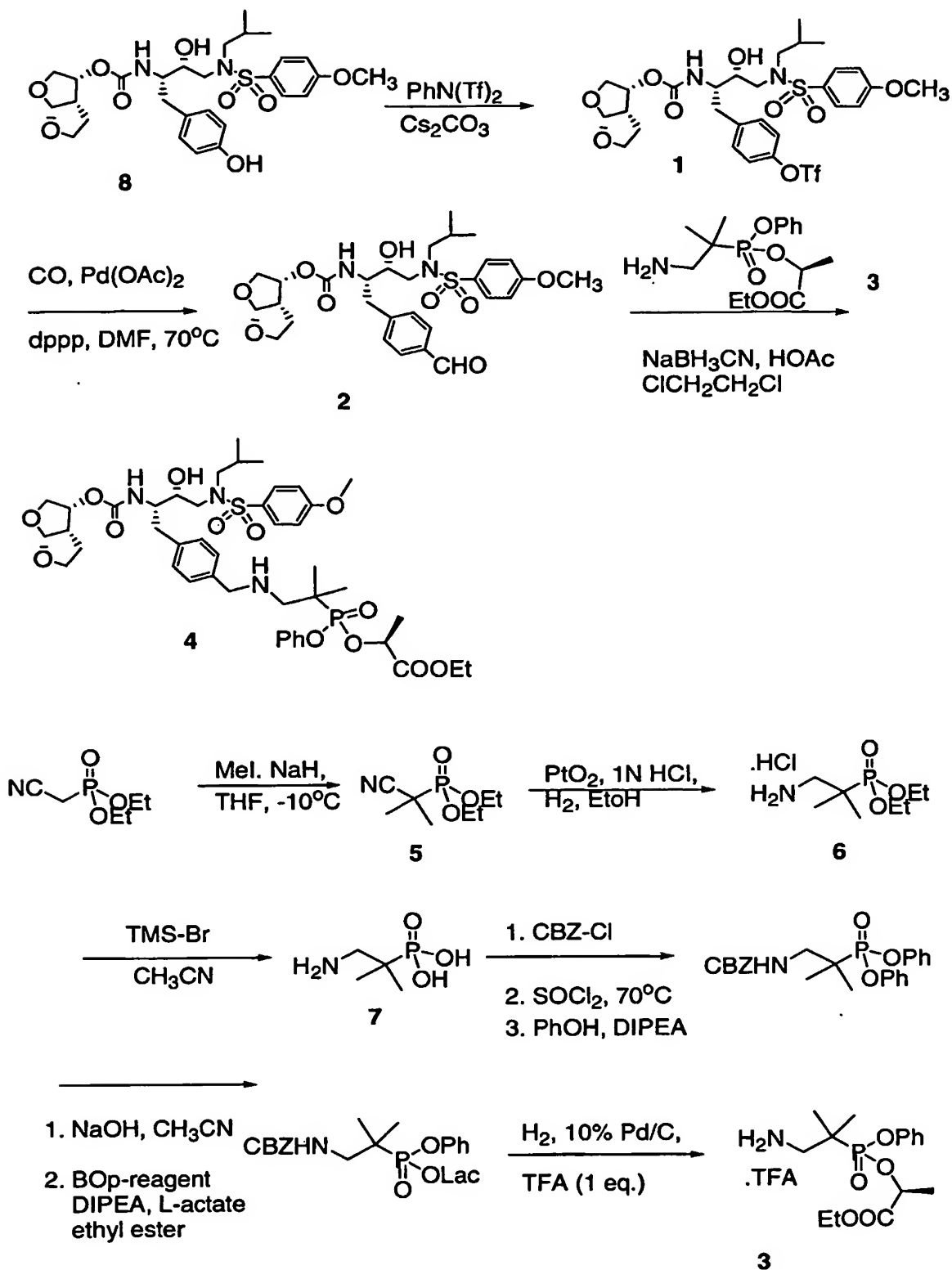
Detection : UV at 260 nm

Temperature : Ambient

Injection : ~ 300 mg on Column

Sample Prep. : Dissolved in ~ 3.5 mL heptane –
ethyl alcohol (70:30)

Retention Times : 35 Minor ~ 14 min
: 35 Major ~ 40 min



Example 29

Triflate derivative 1: A THF-CH₂Cl₂ solution (30mL-10 mL) of 8 (4 g, 6.9 mmol), cesium carbonate (2.7 g, 8 mmol), and N-phenyltrifluoromethane sulfonimide (2.8 g, 8 mmol) was reacted overnight. The reaction mixture was worked up, and concentrated to dryness to give crude triflate derivative 1.

Aldehyde 2: Crude triflate 1 (4.5 g, 6.9 mmol) was dissolved in DMF (20 mL), and the solution was degassed (high vacuum for 2 min, Ar purge, repeat 3 times). Pd(OAc)₂ (0.12 g, 0.27 mmol), and bis(diphenylphosphino)propane (dppp, 0.22 g, 0.27 mmol) were added, the solution was heated to 70°C. Carbon monoxide was rapidly bubbled through the solution, then under 1 atmosphere of carbon monoxide. To this solution were slowly added TEA (5.4 mL, 38 mmol), and triethylsilane (3 ml), 18 mmol). The resulting solution was stirred overnight at room temperature. The reaction mixture was worked up, and purified on silica gel column chromatograph to afford aldehyde 2 (2.1 g, 51 %). (Hostetler, et al J. Org. Chem., 1999. 64, 178-185).

Lactate prodrug 4: Compound 4 is prepared as described above procedure for Example 9E, Compound 35 by the reductive amination between 2 and 3 with NaBH₃CN in 1,2-dichloroethane in the presence of HOAc.

Example 30 Preparation of Compound 3

Diethyl (cyano(dimethyl)methyl) phosphonate 5: A THF solution (30 mL) of NaH (3.4 g of 60% oil dispersion, 85 mmol) was cooled to -10°C, followed by the addition of diethyl (cyanomethyl)phosphonate (5g, 28.2 mmol) and iodomethane (17 g, 112 mmol). The resulting solution was stirred at -10°C for 2 hr, then 0°C for 1 hr, was worked up, and purified to give dimethyl derivative 5 (5 g, 86 %).

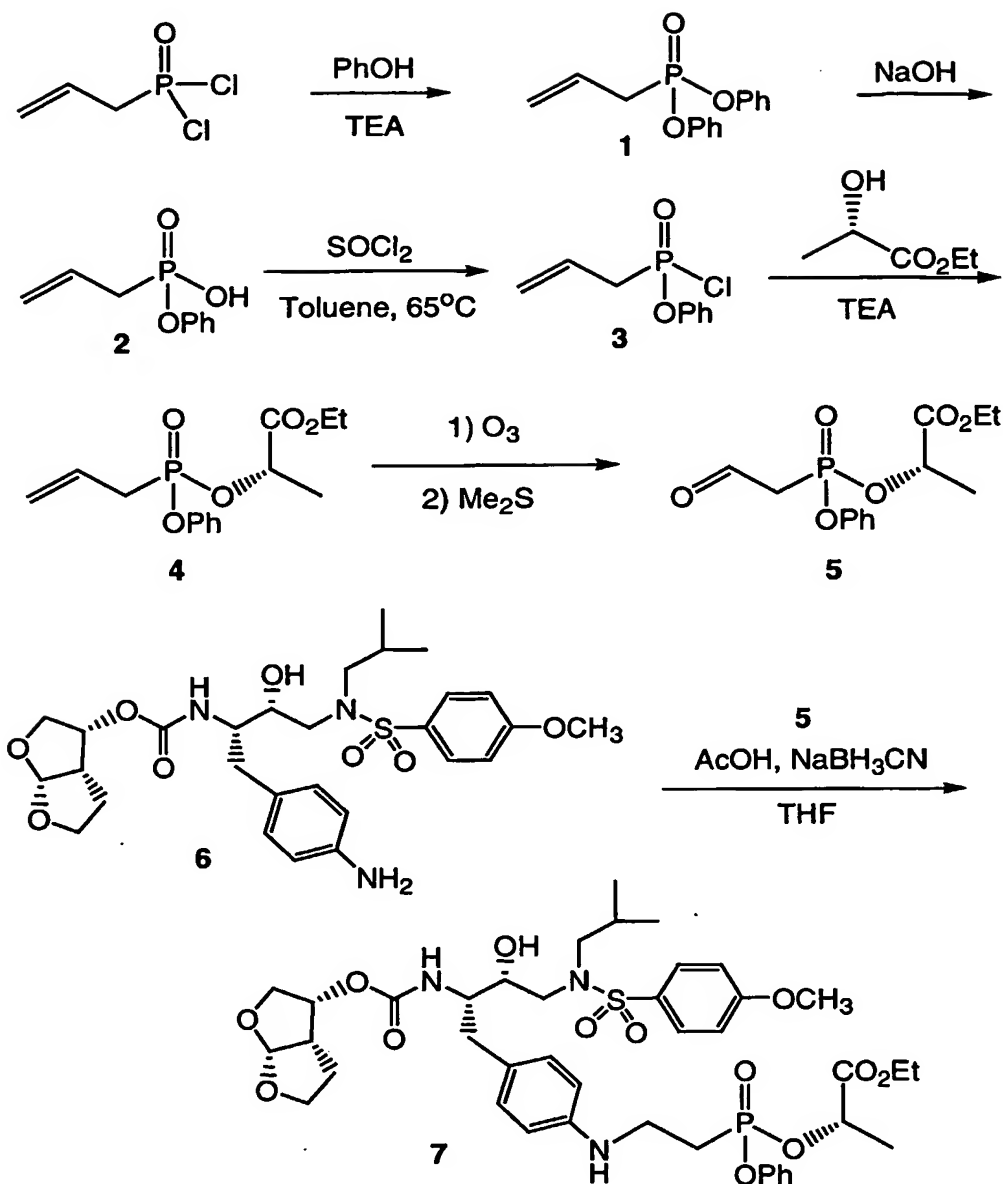
Diethyl (2-amino-1,1-dimethyl-ethyl)phosphonate 6: Compound 5 was reduced to amine derivative 6 by the described procedure (J. Med. Chem. 1999, 42, 5010-5019).

A solution of ethanol (150 mL) and 1N HCl aqueous solution (22 mL) of 5 (2.2 g, 10.7 mmol) was hydrogenated at 1 atmosphere in the presence of PtO₂ (1.25 g) at room temperature overnight. The catalyst was filtered through a celite pad. The filtrate was concentrated to dryness, to give crude 6 (2.5g, as HCl salt).

2-Amino-1,1-dimethyl-ethyl phosphonic acid **7**: A solution of CH₃CN (30 mL) of crude **6** (2.5 g) was cooled to 0°C, and treated with TMSBr (8 g, 52 mmol) for 5 hr. The reaction mixture was stirred with methanol for 1.5 hr at room temperature, concentrated, recharged with methanol, concentrated to dryness to give crude **7** which was used for next reaction without further purification.

Lactate phenyl (2-amino-1,1-dimethyl-ethyl)phosphonate **3**: Compound **3** is synthesized according to the procedures described in Example 9D, Compound 34 for the preparation of lactate phenyl 2-aminoethyl phosphonate 34. Compound **7** is protected with CBZ, followed by the reaction with thionyl chloride at 70°C. The CBZ protected dichlorodate is reacted phenol in the presence of DIPEA. Removal of one phenol, follow by coupling with ethyl L-lactate leads N-CBZ-2-amino-1,1-dimethyl-ethyl phosphonate derivative. Hydrogenation of N-CBZ derivative at 1 atmosphere in the presence of 10 % Pd/C and 1 eq. of TFA affords compound **3** as TFA salt.

Scheme 1

5 Example 1

Monophenol Allylphosphonate 2: To a solution of allylphosphonic dichloride (4 g, 25.4 mmol) and phenol (5.2 g, 55.3 mmol) in CH_2Cl_2 (40 mL) at 0°C was added TEA (8.4 mL, 60 mmol). After stirred at room temperature for 1.5 h, the mixture was diluted with hexane-ethyl acetate and washed with HCl (0.3 N) and water. The organic phase was dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was filtered through a pad of silica gel (eluted with 2:1 hexane-ethyl acetate) to afford crude product diphenol

allylphosphonate **1** (7.8 g, containing the excessive phenol) as an oil which was used directly without any further purification. The crude material was dissolved in CH₃CN (60 mL), and NaOH (4.4N, 15 mL) was added at 0°C. The resulted mixture was stirred at room temperature for 3 h, then neutralized with acetic acid to pH = 8 and concentrated under reduced pressure to remove most of the acetonitrile. The residue was dissolved in water (50 mL) and washed with CH₂Cl₂ (3X25 mL). The aqueous phase was acidified with concentrated HCl at 0°C and extracted with ethyl acetate. The organic phase was dried over MgSO₄, filtered, evaporated and co-evaporated with toluene under reduced pressure to yield desired monophenol allylphosphonate **2** (4.75 g, 95%) as an oil.

Example 2

Monolactate Allylphosphonate **4**: To a solution of monophenol allylphosphonate **2** (4.75 g, 24 mmol) in toluene (30 mL) was added SOCl₂ (5 mL, 68 mmol) and DMF (0.05 mL). After stirred at 65°C for 4 h, the reaction was completed as shown by ³¹P NMR. The reaction mixture was evaporated and co-evaporated with toluene under reduced pressure to give monochloride **3** (5.5 g) as an oil. To a solution of chloride **3** in CH₂Cl₂ (25 mL) at 0°C was added ethyl (s)-lactate (3.3 mL, 28.8 mmol), followed by TEA. The mixture was stirred at 0°C for 5 min then at room temperature for 1 h, and concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N), the organic phase was washed with water, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired monolactate **4** (5.75 g, 80%) as an oil (2:1 mixture of two isomers): ¹H NMR (CDCl₃) δ 7.1-7.4 (m, 5H), 5.9 (m, 1H), 5.3 (m, 2H), 5.0 (m, 1H), 4.2 (m, 2H), 2.9 (m, 2H), 1.6; 1.4 (d, 3H), 1.25 (m, 3H); ³¹P NMR (CDCl₃) δ 25.4, 23.9.

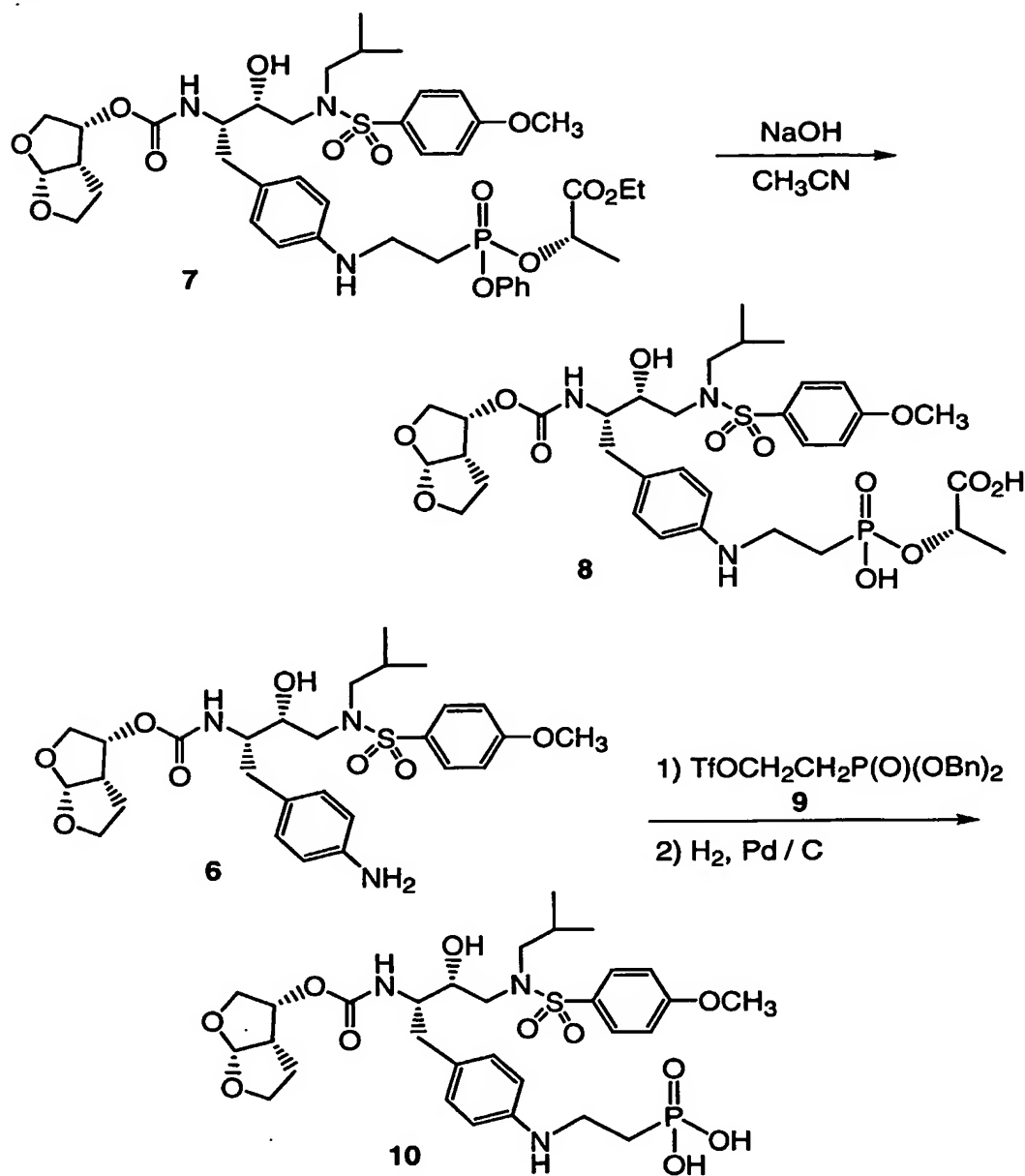
Example 3

Aldehyde **5**: A solution of allylphosphonate **4** (2.5 g, 8.38 mmol) in CH₂Cl₂ (30 mL) was bubbled with ozone air at -78°C until the solution became blue, then bubbled with nitrogen until the blue color disappeared. Methyl sulfide (3 mL) was added at -78°C. The mixture was warmed up to room temperature, stirred for 16 h and concentrated under reduced pressure to give desired aldehyde **5** (3.2 g, as a 1:1 mixture of DMSO): ¹H NMR (CDCl₃) δ 9.8 (m, 1H), 7.1-7.4 (m, 5H), 5.0 (m, 1H), 4.2 (m, 2H), 3.4 (m, 2H), 1.6; 1.4 (d, 3H), 1.25 (m, 3H); ³¹P NMR (CDCl₃) δ 17.7, 15.4.

Example 4

Compound 7: To a solution of aniline 6 (reported before) (1.62 g, 2.81 mmol) in THF (40 mL) was added acetic acid (0.8 mL, 14 mmol), followed by aldehyde 5 (1.3 g, 80%, 3.46 mmol) and MgSO₄ (3 g). The mixture was stirred at room temperature for 0.5 h, then NaBH₃CN (0.4 g, 6.37 mmol) was added. After stirred for 1 h, the reaction mixture was filtered. The filtrate was diluted with ethyl acetate and washed with NaHCO₃, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to give compound 6 (1.1g, 45%) as a 3:2 mixture of two isomers, which were separated by HPLC (mobile phase, 70% CH₃CN/H₂O; flow rate: 70 mL/min; detection: 254 nm; column: 8μ C18, 41X250 mm, Varian). Isomer A (0.39 g): ¹H NMR (CDCl₃) δ 7.75 (d, 2H), 7.1-7.4 (m, 5H), 7.0 (m, 4H), 6.6 (d, 2H), 5.65 (d, 1H), 5.05 (m, 2H), 4.9 (d, 1H), 4.3 (brs, 1H), 4.2 (q, 2H), 3.5-4.0 (m, 6H), 3.9 (s, 3H), 2.6-3.2 (m, 9H), 2.3 (m, 2), 1.6-1.9 (m, 5H), 1.25 (t, 3H), 0.9 (2d, 6H); ³¹P NMR (CDCl₃) δ 26.5; MS (ESI): 862 (M+H). Isomer B (0.59 g): ¹H NMR (CDCl₃) δ 7.75 (d, 2H), 7.1-7.4 (m, 5H), 7.0 (m, 4H), 6.6 (d, 2H), 5.65 (d, 1H), 5.05 (m, 2H), 4.9 (d, 1H), 4.5 (brs, 1H), 4.2 (q, 2H), 3.5-4.0 (m, 6H), 3.9 (s, 3H), 2.7-3.2 (m, 9H), 2.4 (m, 2), 1.6-1.9 (m, 2H), 1.4 (d, 3H), 1.25 (t, 3H), 0.9 (2d, 6H); ³¹P NMR (CDCl₃) δ 28.4; MS (ESI): 862 (M+H).

Scheme 2

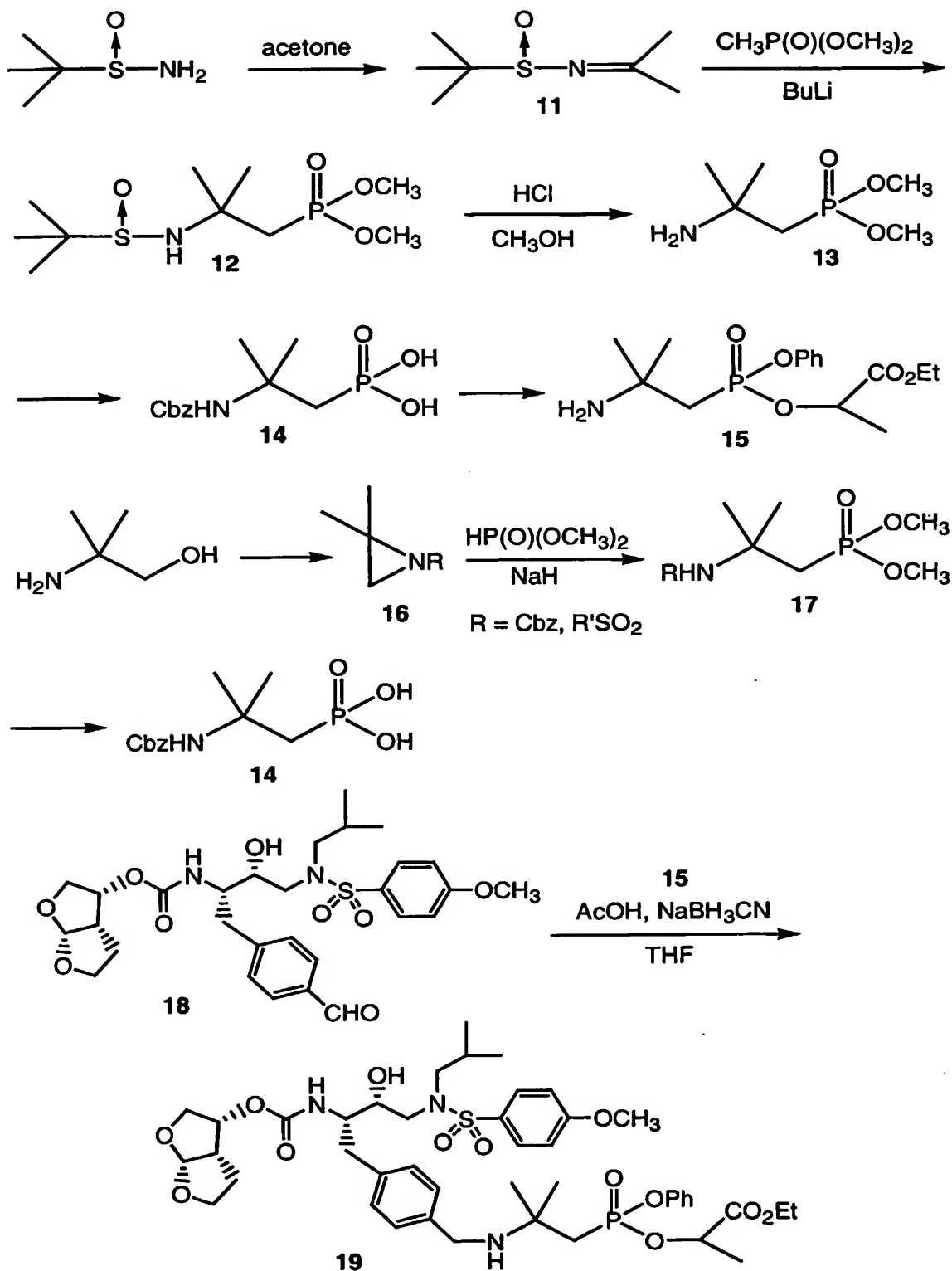
**Example 5**

- 5 Acid 8: To a solution of compound 7 (25 mg, 0.029 mmol) in acetonitrile (1 mL) at 0°C was added NaOH (1N, 0.125 mL). The mixture was stirred at 0°C for 0.5 h and at room temperature for 1 h. The reaction was quenched with acetic acid and purified by HPLC to give acid 8 (10 mg, 45%). ¹H NMR (CD₃OD) δ 7.8 (d, 2H), 7.5 (d, 2H), 7.4 (d, 2H), 7.1 (d, 2H), 5.6 (d, 1H), 4.9 (m, 3H), 3.2-4.0 (m, 6H), 3.9 (s, 3H), 2.6-3.2 (m, 9H), 2.05 (m, 2), 1.4-1.7 (m, 2H), 1.5 (d, 3H), 0.9 (2d, 6H); ³¹P NMR (CD₃OD) δ 20.6; MS (ESI): 758 (M+H).
- 10

Example 6

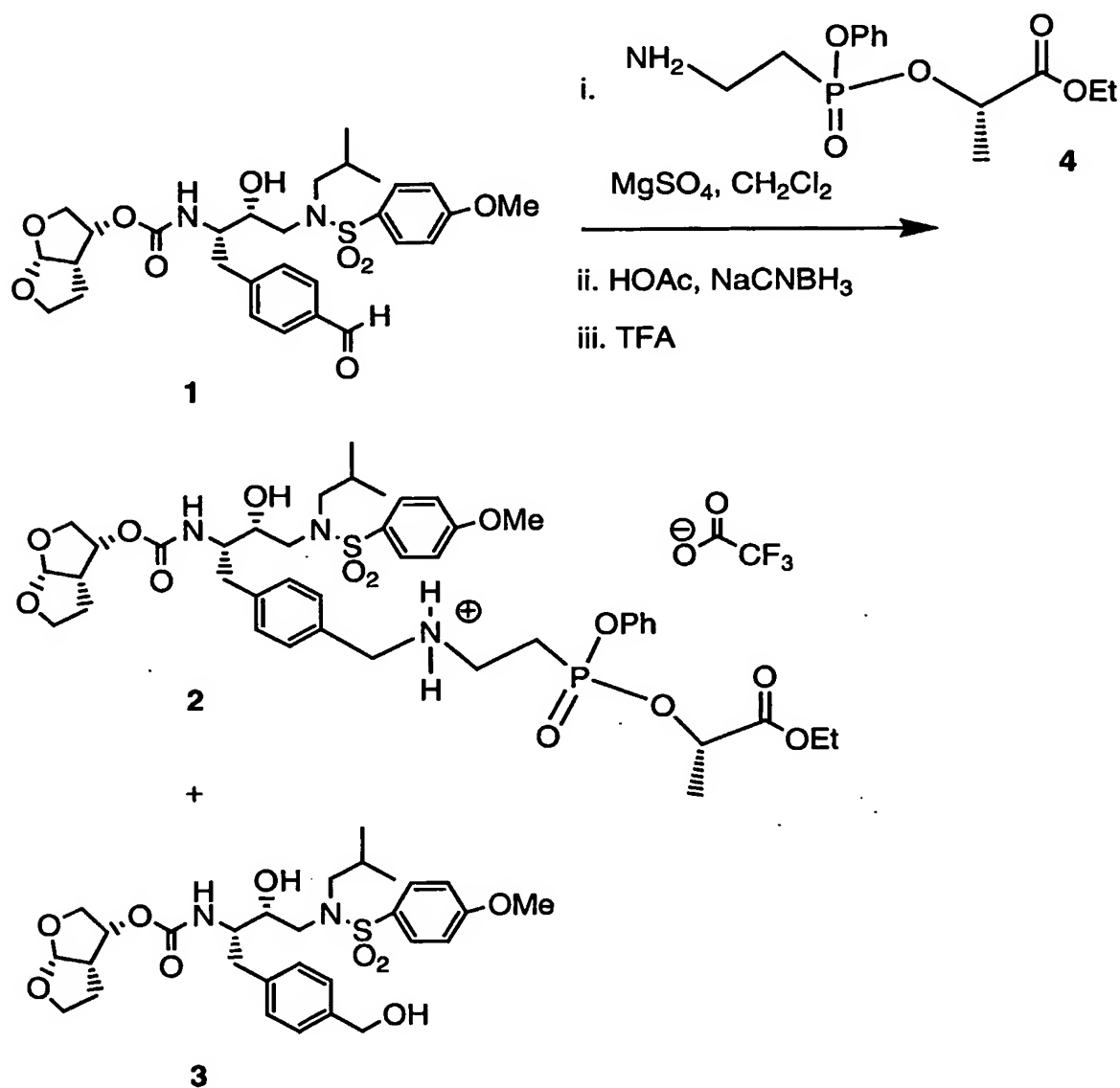
Diacid **10**: To a solution of triflate **9** (94 mg, 0.214 mmol) in CH₂Cl₂ (2 mL) was added a solution of aniline **6** (100 mg, 0.173 mmol) in CH₂Cl₂ (2 mL) at -40°C, followed by 2,6-lutidine (0.026 mL). The mixture was warmed up to room temperature and stirred for 1 h. Cesium carbonate (60 mg) was added and the reaction mixture was stirred for additional 1 h. The mixture was diluted with ethyl acetate, washed with HCl (0.2N), dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by HPLC to afford dibenzyl phosphonate (40 mg). To a solution of this dibenzyl phosphonate in ethanol (3 mL) and ethyl acetate (1 mL) was added 10% Pd/C (40 mg). The mixture was stirred under hydrogen atmosphere (balloon) for 4 h. The reaction mixture was diluted with methanol, filtered and concentrated under reduced pressure. The residue was washed with ethyl acetate and dried to give desired product diacid **10** (20 mg). ¹H NMR (CD₃OD) δ 7.8 (d, 2H), 7.3 (d, 2H), 7.1 (2d, 4H), 5.6 (d, 1H), 4.9 (m, 2H), 3.4-4.0 (m, 6H), 3.9 (s, 3H), 2.5-3.2 (m, 9H), 2.0 (m, 2), 1.4-1.7 (m, 2H), 0.9 (2d, 6H); ³¹P NMR (CD₃OD) δ 22.1; MS (ESI): 686 (M+H).

Scheme 3



The synthesis of compound **19** is outlined in Scheme 3. Condensation of 2-methyl-2-propanesulfonamide with acetone give sulfinyl imine **11** (J. Org. Chem. 1999, 64, 12).

Addition of dimethyl methylphosphonate lithium to **11** afford **12**. Acidic methanolysis of **12** provide amine **13**. Protection of amine with Cbz group and removal of methyl groups yield phosphonic acid **14**, which can be converted to desired **15** using methods reported earlier on. An alternative synthesis of compound **14** is also shown in Scheme 3. Commercially available 2-amino-2-methyl-1-propanol is converted to aziridines **16** according to literature methods (J. Org. Chem. 1992, 57, 5813; and Syn. Lett. 1997, 8, 893). Aziridine opening with phosphite give **17** (Tetrahedron Lett. 1980, 21, 1623). Deprotection (and, if necessary, reprotection) of **17** afford **14**. Reductive amination of amine **15** and aldehyde **18** provides compound **19**.



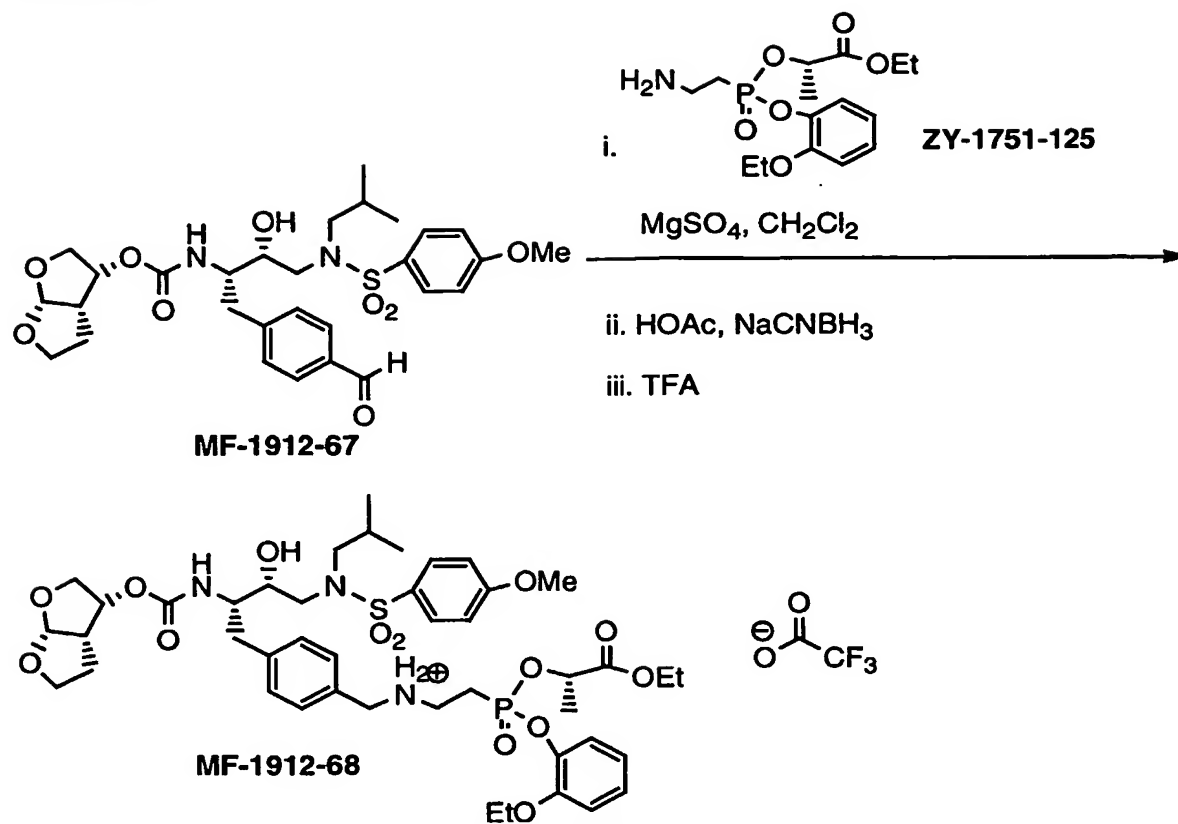
Example 1

2-[[2-(4-{2-(Hexahydro-furo[2,3-b]furan-3-yloxy-carbonylamino)-3-hydroxy-4-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-butyl}-benzylamino)-ethyl]-phenoxy-phosphinoyloxy]-propionic acid ethyl ester **2** (Compound 35, previous Example 9E).

5

A solution of **1** (2.07 g, 3.51 mmol) and **4** (1.33 g, 3.68 mmol of a 4:1 mixture of two diastereomers at the phosphorous center) were dissolved in 14 mL of (CH₂Cl₂)₂ to provide a clear solution. Addition of MgSO₄ (100 mg) to the solution resulted in a white cloudy mixture. The solution was stirred at ambient temperature for 3 hours when acetic acid (0.80 mL, 14.0 mmol) and sodium cyanoborohydride (441 mg, 7.01 mmol) were added. Following the reaction progress by TLC showed complete consumption of the aldehyde starting materials in 1 hour. The reaction mixture was worked up by addition of 200 mL of saturated aqueous NaHCO₃ and 400 mL of CH₂Cl₂. The aqueous layer was extracted with CH₂Cl₂ two more times (2 x 300 mL). The combined organic extracts were dried *in vacuo* and purified by column chromatography (EtOAc- 10% MeOH: EtOAc) to provide the desired product as a foam. The early eluting compound from the column was collected and characterized as alcohol **3** (810 mg, 39%). Addition of TFA (3 x 1 mL) generated the TFA salt which was lyophilized from 50 mL of a 1:1 CH₃CN: H₂O to provide 1.63 g (47%) of the product **2** as a white powder. ¹H NMR (CD₃CN) δ 8.23 (br s, 2H), 7.79 (d, *J*= 8.4 Hz, 2H), 7.45- 7.13 (m, 9H), 7.09 (d, *J*= 8.4 Hz, 2H), 5.86 (d, *J*= 9.0 Hz, 1H), 5.55 (d, *J*= 4.8 Hz, 1H), 5.05-4.96 (m, 1H), 4.96- 4.88 (m, 1H), 4.30-4.15 (m, 4H), 3.89 (s, 3H), 3.86- 3.76 (m, 4H), 3.70- 3.59 (m, 4H), 3.56- 3.40 (m, 2H), 3.34 (d, *J*= 15 Hz, 1H), 3.13 (d, *J*= 13.5 Hz, 1H), 3.06- 2.93 (m, 2H), 2.92- 2.80 (m, 2H), 2.69- 2.43 (m, 3H), 2.03- 1.86 (m, 1H), 1.64- 1.48 (m, 1H), 1.53 and 1.40 (d, *J*= 6.3 Hz, *J*= 6.6 Hz, 3H), 1.45- 1.35 (m, 1H), 1.27 and 1.23 (t, *J*= 6.9 Hz, *J*= 7.2 Hz, 3H), 0.90 (t, *J*= 6.9 Hz, 6H). ³¹P NMR (CD₃CN) δ 24.47, 22.86. ESI (M+ H)⁺ 876.4.

25

Example 2

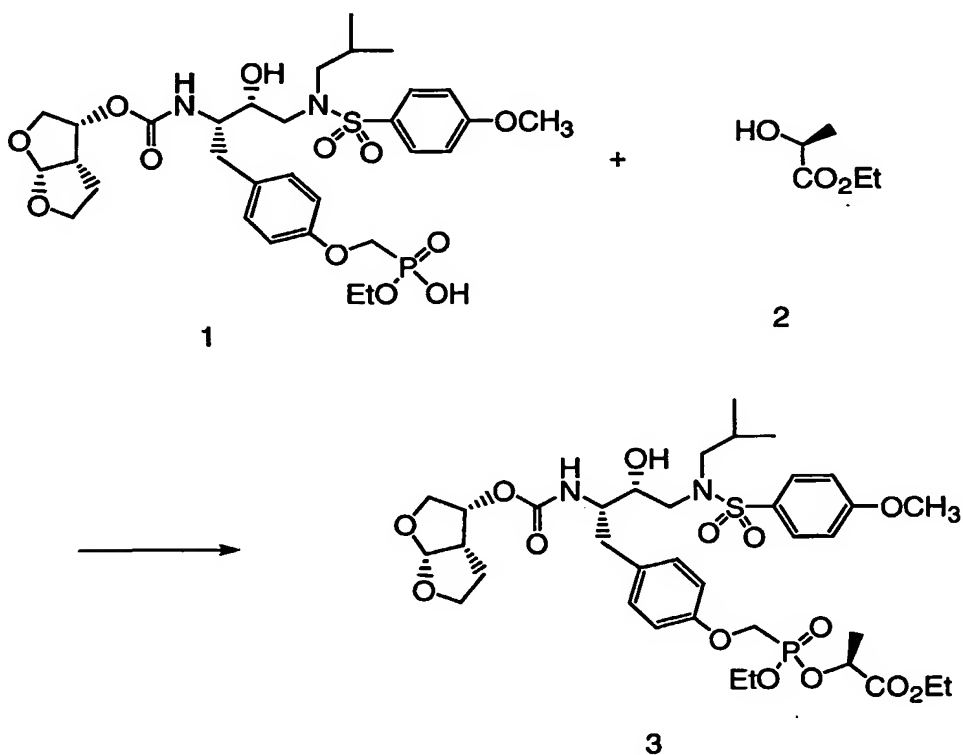
2-{{2-(4-{2-(Hexahydro-furo[2,3-b]furan-3-yloxy-carbonylamino)-3-hydroxy-4-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-butyl}-benzylamino)-ethyl]-phenoxy-phosphinoyloxy}-propionic acid ethyl ester (MF-1912-68):

A solution of MF-1912-67 (0.466 g, 0.789 mmol) and ZY-1751-125 (0.320 g, 0.789 mmol of a 1:1 mixture of two diastereomers at the phosphorous center) were dissolved in 3.1 mL of $(\text{CH}_2\text{Cl}_2)_2$ to provide a clear solution. Addition of MgSO_4 (20 mg) to the solution resulted in a white cloudy mixture. The solution was stirred at ambient temperature for 3 hours when acetic acid (0.181 mL, 3.16 mmol) and sodium cyanoborohydride (99 mg, 1.58 mmol) were added. Following the reaction progress by TLC showed complete consumption of the aldehyde starting materials in 1.5 hour. The reaction mixture was worked up by addition of 50 mL of saturated aqueous NaHCO_3 and 200 mL of CH_2Cl_2 . The aqueous layer was extracted with CH_2Cl_2 two more times (2 x 200 mL). The combined organic extracts were dried *in vacuo* and purified by column chromatography (EtOAc- 10% MeOH: EtOAc) to provide the desired product as a foam. The early eluting compound from the column was collected and characterized to be MF-1912-48b alcohol (190 mg, 41%). Addition of TFA (3 x 1 mL) generated the TFA salt which was lyophilized from 50 mL of a 1:1 CH_3CN : H_2O to

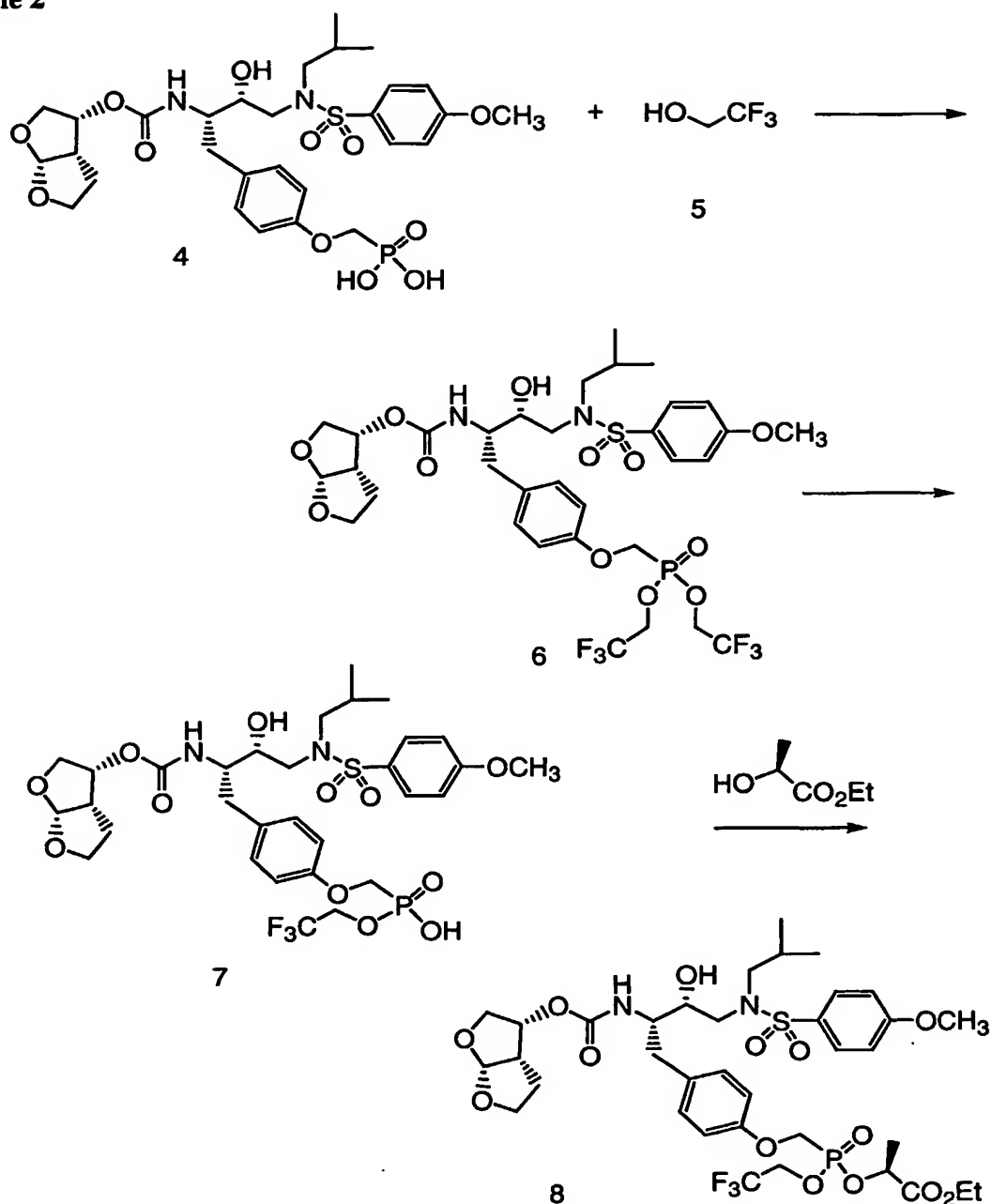
provide 0.389 g (48%) of the product as a white powder. ^1H NMR (CD_3CN) δ 8.39 (br s, 2H), 7.79 (d, $J=8.7$ Hz, 2H), 7.40 (d, $J=7.5$ Hz, 2H), 7.34 (d, $J=8.1$ Hz, 2H), 7.26-7.16 (m, 2H), 7.10 (d, $J=9$ Hz, 3H), 7.01- 6.92 (m, 1H), 5.78 (d, $J=9.0$ Hz, 1H), 5.55 (d, $J=5.1$ Hz, 1H), 5.25-5.03 (m, 1H), 4.95- 4.88 (m, 1H), 4.30- 4.17 (m, 4H), 4.16- 4.07 (m, 2H), 3.90 (s, 3H), 3.88-3.73 (m, 4H), 3.72- 3.60 (m, 2H), 3.57- 3.38 (m, 2H), 3.32 (br d, $J=15.3$ Hz, 1H), 3.13 (br d, $J=14.7$ Hz, 1H), 3.05- 2.92 (m, 2H), 2.92- 2.78 (m, 2H), 2.68- 2.48 (m, 3H), 2.03- 1.90 (m, 1H), 1.62- 1.51 (m, 1H), 1.57 and 1.46 (d, $J=6.9$ Hz, $J=6.9$ Hz, 3H), 1.36- 1.50 (m, 1H), 1.43- 1.35 (m, 4H), 1.33- 1.22 (m, 3H), 0.91 (t, $J=6.6$ Hz, 6H). ^{31}P NMR (CD_3CN) δ 25.27, 23.56. ESI ($\text{M}+\text{H}$) $^+$ 920.5.

10

Scheme 1



Scheme 2



Example 1

- 5 Mono-Ethyl mono-lactate 3: To a solution of 1 (96mg, 0.137 mmol) and ethyl lactate 2 (0.31 mL, 2.7 mmol) in pyridine (2 mL) was added N, N-dicyclohexylcarbodiimide (170 mg, 0.822 mmol). The solution was stirred for 18h at 70°C. The mixture was cooled to room temperature and diluted with dichloromethane. The solid was removed by filtration and the filtrate was concentrated. The residue was suspended in diethyl ether/dichloromethane and

filtered again. The filtrate was concentrated and mixture was chromatographed on silica gel eluting with EtOAc/hexane to provide compound 3 (43 mg, 40%) as a foam: ^1H NMR (CDCl_3) δ 7.71 (d, 2H), 7.00 (d, 2H); 7.00 (d, 2H), 6.88 (d, 2H), 5.67 (d, 1H), 4.93-5.07 (m, 2H), 4.15-4.39 (m, 6H), 3.70-3.99 (m, 10H), 2.76-3.13 (m, 7H), 1.55-1.85 (m, 9H), 1.23-1.41 (m, 6H), 0.90 (dd, 6H); ^{31}P NMR (CDCl_3) δ 19.1, 20.2; MS (ESI) 823 (M+Na).

Example 2

Bis-2,2,2-trifluoroethyl phosphonate 6: To a solution of 4 (154mg, 0.228 mmol) and 222,-trifluoroethanol 5 (1 mL, 13.7 mmol) in pyridine (3 mL) was added N, N-dicyclohexylcarbodiimide (283 mg, 1.37 mmol). The solution was stirred for 6.5h at 70°C. The mixture was cooled to room temperature and diluted with dichloromethane. The solid was removed by filtration and the filtrate was concentrated. The residue was suspended in dichloromethane and filtered again. The filtrate was concentrated and mixture was chromatographed on silica gel eluting with EtOAc/hexane to provide compound 6 (133 mg, 70%) as a foam: ^1H NMR (CDCl_3) δ 7.71 (d, 2H), 7.21 (d, 2H); 7.00 (d, 2H), 6.88 (dd, 2H), 5.66 (d, 1H), 4.94-5.10 (m, 3H), 4.39-4.56 (m, 6H), 3.71-4.00 (m, 10H), 2.77-3.18 (m, 7H), 1.67-1.83(m, 2H), 0.91 (dd, 4H); ^{31}P NMR (CDCl_3) δ 22.2; MS (ESI) 859 (M+Na).

Example 3

Mono-2,2,2-trifluoroethyl phosphonate 7: To a solution of 6 (930mg, 1.11 mmol) in THF (14 mL) and water (10 mL) was added an aqueous solution of NaOH in water (1N, 2.2 mL). The solution was stirred for 1h at 0°C. An excess amount of Dowex resin (H^+) was added to until pH=1. The mixture was filtered and the filtrate was concentrated under reduced pressure. The concentrated solution was azeotroped with EtOAc/toluene three times and the white powder was dried *in vacuo* provide compound 7 (830 mg, 100%). ^1H NMR (CDCl_3) δ 7.71 (d, 2H), 7.11 (d, 2H); 6.99 (d, 2H), 6.85 (d, 2H), 5.63 (d, 1H), 5.26 (m, 1H), 5.02 (m, 1H), 4.40 (m, 1H), 4.14 (m, 4H), 3.60-3.95 (m, 12H), 2.62-3.15 (m, 15H), 1.45-1.84 (m, 3H), 1.29 (m, 4H), 0.89 (d, 6H); ^{31}P NMR (CDCl_3) δ 19.9; MS (ESI) 723 (M+Na).

Example 4

Mono-2,2,2-trifluoroethyl mono-lactate 8: To a solution of 7 (754mg, 1 mmol) and N, N-dicyclohexylcarbodiimide (1.237 g, 6 mmol) in pyridine (10 mL) was added ethyl lactate

(2.26 mL, 20 mmol). The solution was stirred for 4.5h at 70°C. The mixture was concentrated and the residue was suspended in diethyl ether (5 mL) and dichloromethane (5 mL) and filtered. The solid was washed a few times with diethyl ether. The combined filtrate was concentrated and the crude product was chromatographed on silica gel, eluting with EtOAc and hexane to provide compound 8 (610 mg, 71%) as a foam. ¹H NMR (CDCl₃) δ 7.71 (d, 2H), 7.16 (d, 2H); 6.99 (d, 2H), 6.88 (dd, 2H), 5.66 (d, 1H), 4.95-5.09 (m, 2H), 4.19-4.65 (m, 6H), 3.71-4.00 (m, 9H), 2.76-3.13 (m, 6H), 1.57-1.85 (m, 7H), 1.24-1.34 (m, 4H), 0.91 (dd, 6H); ³¹P NMR (CDCl₃) δ 20.29, 21.58; MS (ESI) 855 (M+1).

10 Example 1

Boc-protected hydroxylamine 1: A solution of diethyl hydroxymethyl phosphonate triflate (0.582 g, 1.94 mmol) in dichloromethane (19.4 mL) was treated with triethylamine (0.541 mL, 3.88 mmol). Tert-butyl N-hydroxy-carbamate (0.284 g, 2.13 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between dichloromethane and water. The organic phase was washed with saturated NaCl, dried (MgSO₄) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (1/1 – ethyl acetate/hexane) affording the BOC-protected hydroxylamine 1 (0.41 g, 75%) as an oil: ¹H NMR (CDCl₃) δ 7.83 (s, 1H), 4.21 (d, 2H), 4.18 (q, 4H), 1.47 (s, 9H), 1.36 (t, 6H); ³¹P NMR (CDCl₃) δ 19.3.

20

Example 2

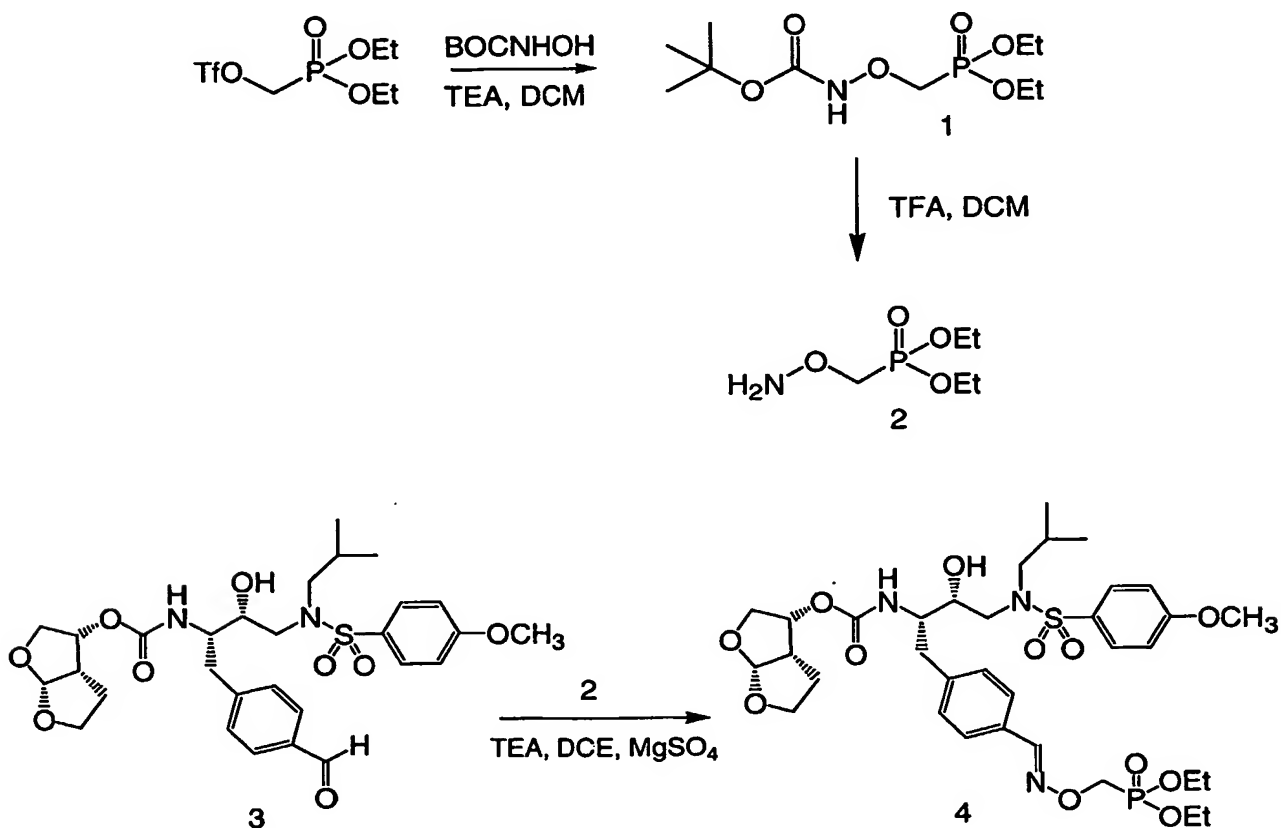
Hydroxylamine 2: A solution of BOC-protected hydroxylamine 1 (0.305 g, 1.08 mmol) in dichloromethane (2.40 mL) was treated with trifluoroacetic acid (0.829 mL, 10.8 mmol). The reaction was stirred for 1.5 hours at room temperature and then the volatiles were evaporated under reduced pressure with toluene to afford the hydroxylamine 2 (0.318 g, 100%) as the TFA salt which was used directly without any further purification: ¹H NMR (CDCl₃) δ 10.87 (s, 2H), 4.45 (d, 2H), 4.24 (q, 4H), 1.38 (t, 6H); ³¹P NMR (CDCl₃) δ 16.9; MS (ESI) 184 (M+H).

30 Example 3

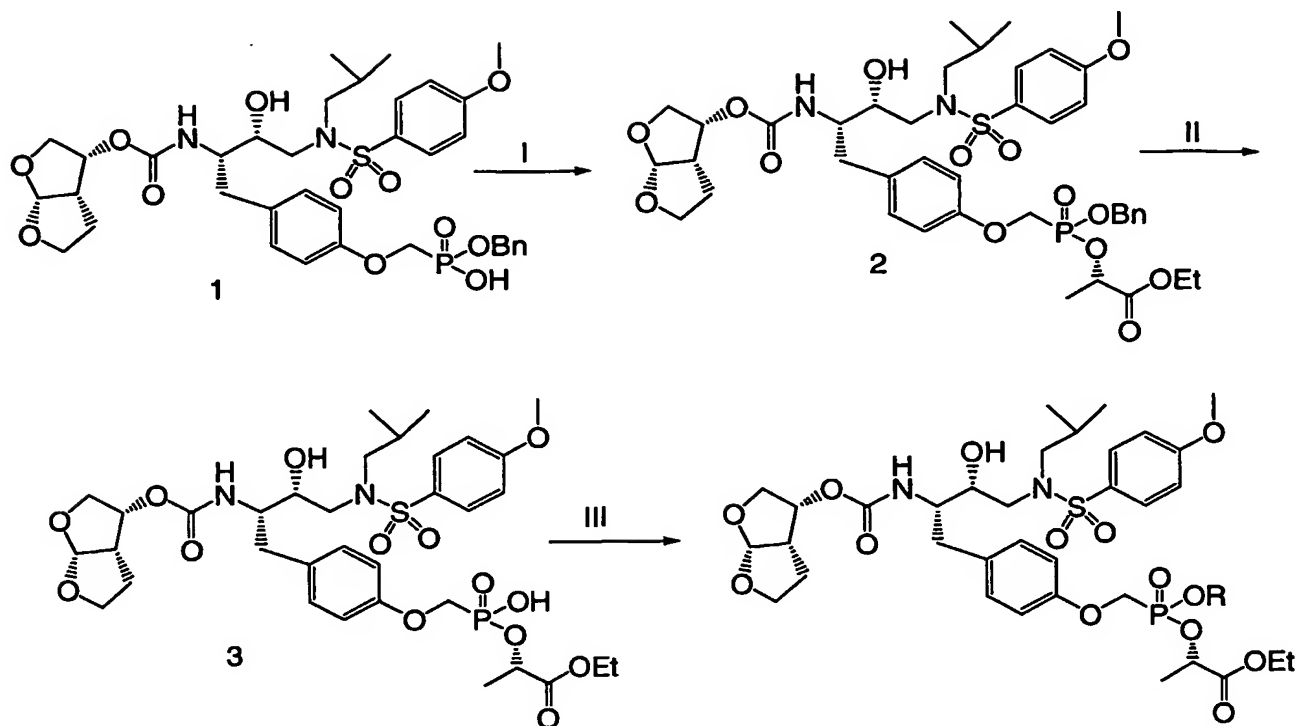
Oxime 4: To a solution of aldehyde 3 (96 mg, 0.163 mmol) in 1,2-dichloroethane (0.65 mL) was added hydroxylamine 2 (72.5 mg, 0.244 mmol), triethylamine (22.7 μL, 0.163 mmol) and MgSO₄ (10 mg). The reaction mixture was stirred at room temperature for 2 hours then

the mixture was partitioned between dichloromethane and water. The organic phase was washed with saturated NaCl, dried (MgSO₄) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (90/10 – ethyl acetate/hexane) affording, GS-277771, oxime 4 (0.104 g, 85%) as a solid: ¹H NMR (CDCl₃) δ 8.13 (s, 1H), 7.72 (d, 2H), 7.51 (d, 2H), 7.27 (d, 2H), 7.00 (d, 2H), 5.67 (d, 1H), 5.02 (m, 2H), 4.54 (d, 2H), 4.21 (m, 4H), 3.92 (m, 1H), 3.89 (s, 3H), 3.88 (m, 1H), 3.97-3.71 (m, 2H), 3.85-3.70 (m, 2H), 3.16-2.99 (m, 2H), 3.16-2.81 (m, 7H), 1.84 (m, 1H), 1.64-1.48 (m, 2H), 1.37 (t, 6H), 0.94-0.90 (dd, 6H); ³¹P NMR (CDCl₃) δ 20.0; MS (ESI) 756 (M+H).

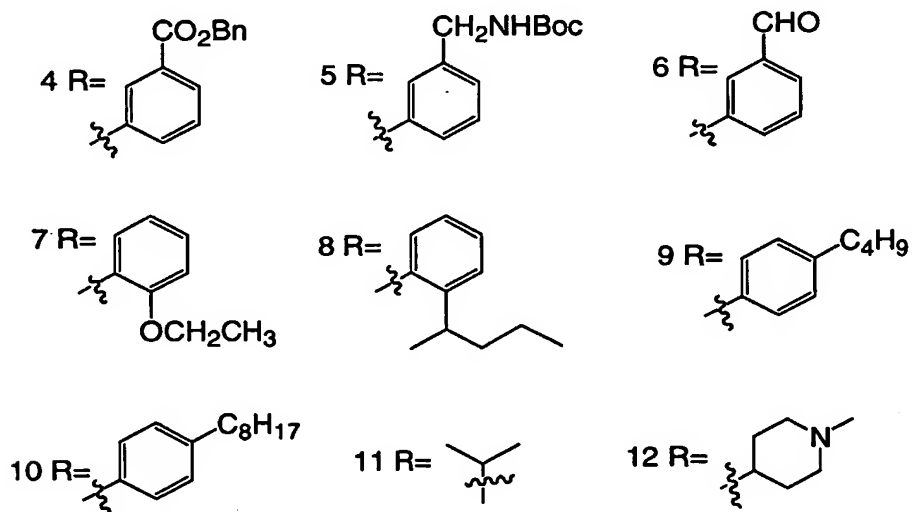
10 Scheme 1



Scheme 1



I. Ethyl(S)-(-)lactate/Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/ DIPEA/EtOAc; II. H_2 /20%Pd-C/EtOAc-EtOH; III. ROH/Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/ DIPEA/EtOAc



Example 1

Compound 1 was prepared according to methods from previous Schemes

Example 2

- 5 Compound 2: To a solution of compound 1 (5.50 g, 7.30 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (5.70g, 10.95 mmol), and Ethyl(S)-(-)lactate (1.30 g, 10.95 mmol) in DMF (50 mL) was added Diisopropylethylamine (5.08 mL, 29.2 mmol). The mixture was stirred for 7 hours after which was diluted in EtOAc. The organic phase was washed with H₂O (5X), brine, dried over MgSO₄ and *concentrated in*
10 *vacuo*. The residue was purified by silica gel chromatography (CH₂Cl₂/Isopropanol= 100/4) to give 3.45 g of compound 2.

Example 3

- Compound 3: To the mixture of compound 2 (3.45 g) in EtOH/EtOAc (300 mL/100 mL) was
15 added 20% Pd/C (0.700 g). The mixture was hydrogenated for 1 hour. Celite was added and the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and washed with ethanol. Concentration gave 2.61 g of compound 3.

Example 4

- 20 Compound 4: To a solution of compound 3 (1.00 g, 1.29 mmol) in dry dimethylformamide (5 mL) was added 3-Hydroxy-benzoic acid benzyl ester (0.589 g, 2.58 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.34 g, 2.58 mmol), followed by addition of Diisopropylethylamine (900 μ L, 5.16 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over
25 sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH₂Cl₂/Isopropanol= 100/3) to provide 67.3 mg of compound 4: ¹H NMR (CDCl₃) δ 7.91 (2H,d, J=8.9 Hz), 7.75 (2H, m), 7.73-7.3 (13H,m), 7.25 (2H, m), 7.21-6.7(6H, m), 5.87(1H, m), 5.4-4.8(6H, m), 4.78-4.21 (4H, m), 3.98 (3H,s), 2.1-1.75 (8H, m), 1.55 (3H, m), 1.28(3H, m), 0.99(6H, m).

30

Example 5

Compound 5: To a solution of compound 3 (1.40 g, 1.81 mmol) in dry dimethylformamide (5 mL) was added (4-Hydroxy-benzyl)-carbamic acid tert-butyl ester (0.80 g, 3.62 mmol),

Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.74 g, 3.62 mmol), followed by addition of Diisopropylethylamine (1.17 ml, 7.24 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue
5 was purified by silica gel chromatography ($\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/3.5$) to provide 770 mg of compound 5: ^1H NMR (CDCl_3) δ 7.8(2H, d, $J=8.9\text{Hz}$), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-5.1(2H, m), 4.6-4.23 (4H,m), 3.98 (3H, s), 3.7-2.6 (15H, m), 2.2-1.8 (12H, m), 1.72 (3H, s), 1.58(3H, m), 1.25 (3H, m), 0.95 (6H, m).

10 Example 6

Compound 6: To a solution of compound 3 (1.00 g, 1.29 mmol) in dry dimethylformamide (6 mL) was added 3-Hydroxybenzaldehyde (0.320 g, 2.60 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.35 g, 2.60 mmol), followed by addition of Diisopropylethylamine (901 μL , 5.16 mmol). The mixture was stirred for 14
15 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ($\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/5$) to provide 880 mg of compound 6.

20 Example 7

Compound 7: To a solution of compound 3 (150 mg, 0.190 mmol) in dry dimethylformamide (1 mL) was added 2-Ethoxy-phenol (48.0 μL , 0.380 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (198 mg, 0.380 mmol), followed by addition of Diisopropylethylamine (132 μL , 0.760 mmol). The mixture was
25 stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ($\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/4$) to provide 84.7 mg of compound 7: ^1H NMR (CDCl_3) δ 7.73 (2H, d, $J=8.9\text{ Hz}$), 7.15 (2H, m), 7.01-6.9 (8H, m), 5.66 (1H, m), 5.22-5.04 (2H, m), 4.56- 4.2 (6H, m), 4.08 (2H, m), 3.89 (3H, m), 3.85-3.69
30 (6H, m), 3.17-2.98 (7H, m), 2.80(3H, m) 1.86 (1H, m), 1.65(2H, m), , 1.62-1.22 (6H, m), 0.92(6H, m).

Example 8

Compound 8: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 2-(1-methylbutyl) phenol (21.2 mg, 0.130 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition of Diisopropylethylamine (45.0 μ L, 0.260 mmol). The mixture
5 was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 8.20 mg of compound 8: ^1H NMR (CDCl_3) δ 7.73 (2H, d, $J=8.9$ Hz), 7.25 (2H, m), 7.21-6.89 (8H, m), 5.7(1H, m), 5.29-4.9 (2H, m), 4.56- 4.2 (6H, m), 3.89 (3H, m), 3.85-3.69 (6H, m), 3.17-2.89 (8H, m), 2.85(3H, m), 2.3-
10 1.65(4H, m), 1.55-1.35 (6H, m), 0.92(6H, m).

Example 9

Compound 9: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 4-N-Butylphenol (19.4 mg, 0.130 mmol),
15 Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition (45.0 μ L, 0.260 mmol) of Diisopropylethylamine. The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 9.61 mg of compound 9: ^1H NMR (CDCl_3)
20 δ 7.8(2H, d, $J=8.9$ Hz), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-4.5 (4H, m), 4.3-3.4.1 (4H, m), 3.9 (3H, m), 3.3-2.59 (11H, m), 2.25 (2H, m), 1.85-1.5 (5H, m), 1.4-1.1(10H, m), 0.95(9H, m).

Example 10

25 Compound 10: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 4-Octylphenol (26.6 mg, 0.130 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition of Diisopropylethylamine (45.0 μ L, 0.260 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over
30 sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 7.70 mg of compound 10: ^1H NMR (CDCl_3) δ 7.75 (2H, d, $J=8.9$ Hz), 7.3 (2H, m), 7.2-6.8 (8H, m), 5.70 (1H, m), 5.3-4.9 (4H, m), 4.6- 3.9 (4H, m),

3.89 (3H, m), 3.85-2.59 (12H, m), 2.18-1.75 (10H, m), 1.69-1.50 (8H, m), 1.4-1.27(6H,m), 0.95(9H, m).

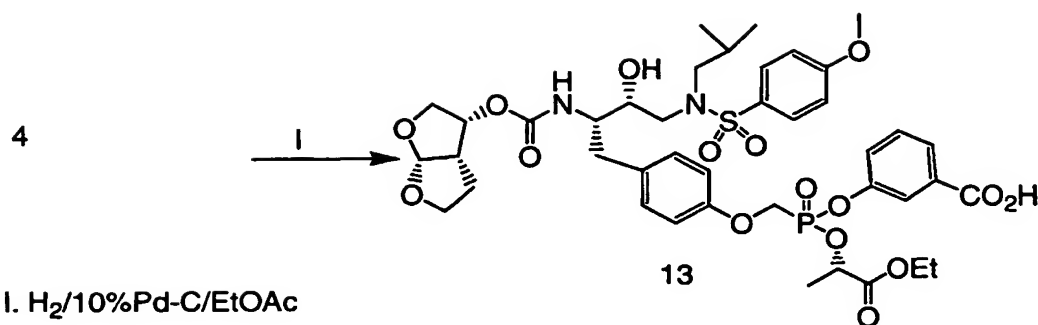
Example 11

5 Compound 11: To a solution of compound 3 (100 mg, 0.120 mmol) in dry dimethylformamide (1 mL) was added Isopropanol (20.0 μ L, 0.240 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (135 mg, 0.240 mmol), followed by addition of Diisopropylethylamine (83.0 μ L, 0.480 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over
10 sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2 /Isopropanol= 100/4) to provide 12.2 mg of compound 11: ^1H NMR (CDCl_3) δ 7.71 (2H, d, J=8.9 Hz), 7.15 (2H, m), 7.0 (2H, m), 6.89 (2H, m), 5.65 (1H, m), 5.03-4.86(4H, m), 4.34-4.19 (3H, m), 3.89 (3H, s), 3.88 (1H, m), 3.82 (2H, m), 3.65 (4H, m), 3.2-2.9 (11H, m), 2.80(3H, m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m),
15 1.30(3H,m), 0.92(6H, m).

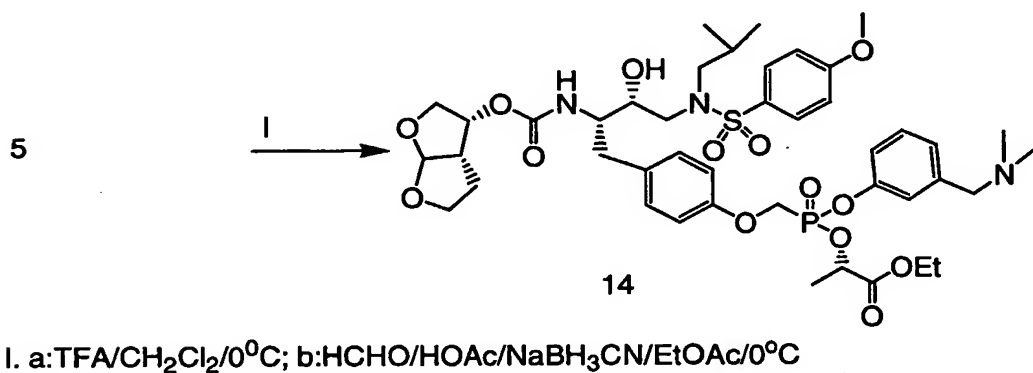
Example 12

Compound 12: To a solution of compound 3 (100 mg, 0.120 mmol) in dry dimethylformamide (1mL) was added 4-Hydroxy-1-methylpiperidine (30.0 mg, 0.240
20 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (135 mg, 0.240 mmol), followed by addition of Diisopropylethylamine (83.0 μ L, 0.480 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 50.1 mg of compound 12: ^1H NMR
25 (CDCl_3) δ 7.73 (2H, d, J=8.9 Hz), 7.18 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 5.67 (1H, m), 5.2-4.9 (4H, m), 4.30-4.11 (4H, m), 3.98 (1H, m), 3.89 (3H, s), 3.87 (1H, m), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (14H, m), 2.80(3H, m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.30(3H,m), 0.92(6H, m).

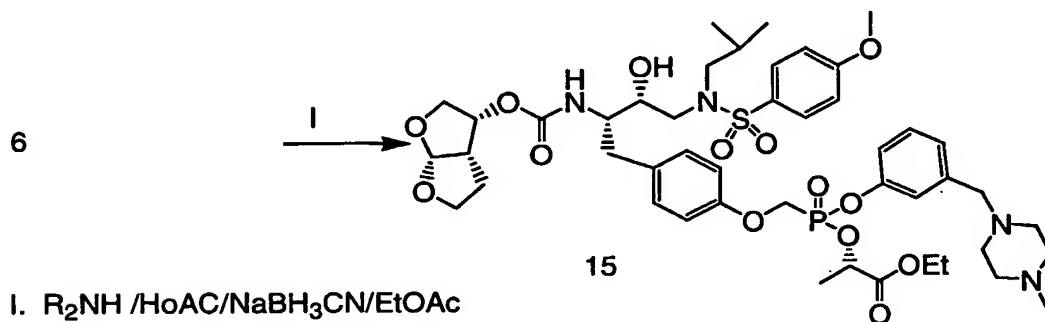
Scheme 2



Scheme 3



Scheme 4



5

Example 13

Compound 13: To a solution of compound 4 (4.9 g) in EtOAc (150ml) was added 20% Pd/C (0.90 g), the reaction mixture was hydrogenated for 1 hour. Celite was added and the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and washed with ethanol. Concentration gave 4.1 g of compound 13: $^1\text{H NMR}$ (CDCl_3) δ 7.91 (2H, d, $J=8.9$ Hz),

7.75 (2H, m), 7.73-7.3 (8H, m), 7.25 (2H, m), 7.21-6.7(6H, m), 5.4-4.8(6H, m), 4.78-4.21 (4H, m), 3.98 (3H,s), 2.1-1.75 (8H, m), 1.55 (3H, m), 1.28(3H, m), 0.99(6H, m).

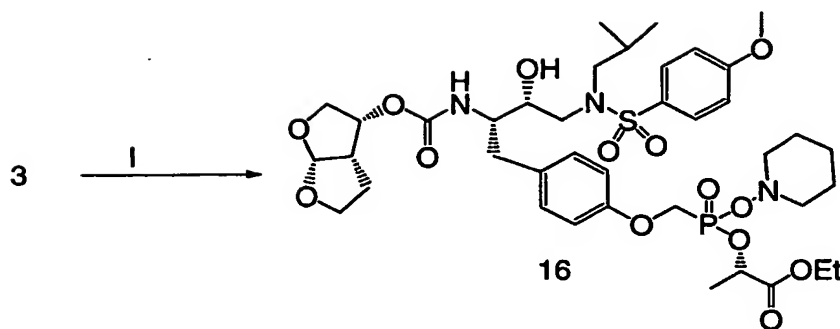
Example 14

- 5 Compound 14: To a solution of compound 5 (0.770 g, 0.790 mmol) in dichloromethane (10 mL), under ice-cooling, was added trifluoroacetic acid (5 mL), the resulting mixture was stirred at 25°C for two hours. The reaction mixture was concentrated under reduced pressure and the residue was co-evaporated with EtOAc to provide an yellow oil. To a solution of the above oil in (10 mL) of EtOAc, under ice-cooling and stirring was added formaldehyde (210
10 μ L, 2.86 mmol), acetic acid (252 μ L, 4.30 mmol), followed by sodium cyanoborohydride (178 mg, 2.86 mmol). The mixture was further stirred at 25°C for 2 hours. The above mixture was concentrated and diluted with EtOAc and washed with H₂O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using reversed-phase HPLC to provide 420 mg of compound 14: ¹H NMR (CDCl₃)
15 δ 7.8(2H, d, J=8.9Hz), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-5.1(2H, m), 4.6-4.23 (4H,m), 3.98 (3H, s), 3.7-2.6 (15H, m), 2.2-1.8 (8H, m), 1.72 (3H, s), 1.58(3H, m), 1.25 (3H, m), 0.95 (6H, m).

Example 15

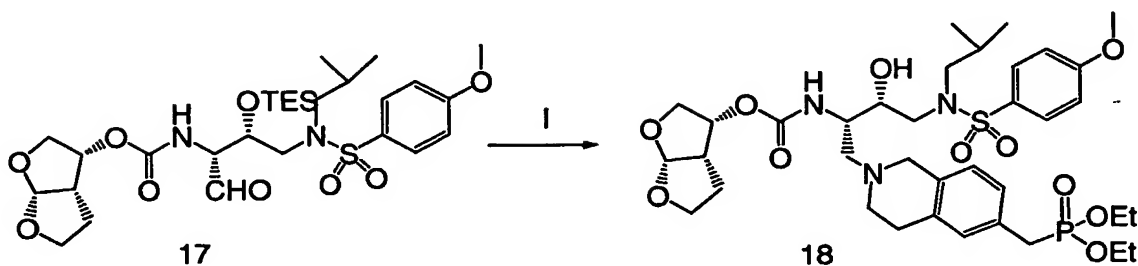
- 20 Compound 15: To a solution of compound 6 (100mg, 0.114 mmol) in EtOAc (1 mL) was added 1-Methyl-piperazine (63.2 mg, 0.570 mmol), acetic acid (34.0 μ l, 0.570 mmol) followed by Sodium Cyanoborohydride (14.3 mg, 0.228mmol). The mixture was stirred at 25°C for 14 hours. The reaction mixture was concentrated and diluted with EtOAc and washed with H₂O (5X), brine (2x), dried over sodium sulfate, filtered, and concentrated under reduced pressure.
25 The residue was purified using silica gel chromatography (CH₂Cl₂/Isopropanol= 100/6.5) to give 5.22 mg of compound 15: ¹H NMR (CDCl₃) δ 7.73 (2H, d, J=8.9 Hz), 7.4-7.18(8H, m), 7.1-6.89 (2H, m), 5.67 (1H, m), 5.2-4.9 (4H, m), 4.30-4.11 (4H, m), 3.98 (1H, m), 3.89 (3H, s), 3.87 (1H, m), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80-2.25 (8H,m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.30(3H,m), 0.92(6H, m).

Scheme 5



I. Piperidin-1-ol/DCC/Pyridine

Scheme 6

I. a: R₂NH /HOAc/NaBH₃CN/EtOAc b: 2%HF/CH₃CN

Example 16

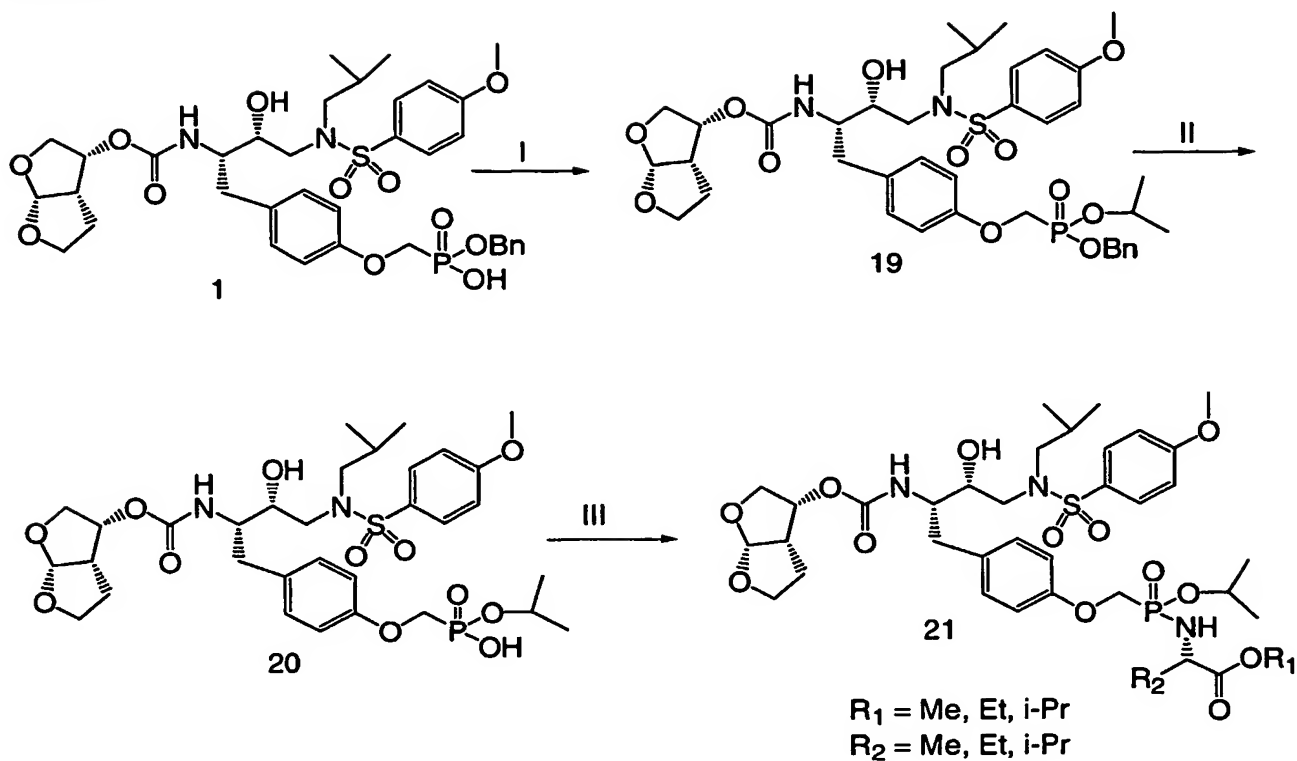
Compound 16: To a solution of compound 3 (100mg, 0.120 mmol) in Pyridine (600 μ L) was added Piperidin-1-ol (48.5 mg, 0.480 mmol), followed by N,N-Dicyclohexylcarbodiimide (99.0 mg, 0.480 mmol). The mixture was stirred for 6 hours, the solvent was concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH₂Cl₂/Methanol= 100/5) to provide 17 mg of compound 16: ¹H NMR (CDCl₃) δ 7.73 (2H, d, J=8.9 Hz), 7.16 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 5.68 (1H, m), 5.17 (1H, m), 5.04 (1H, m), 4.5-4.2 (4H, m), 3.90 (3H, s), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80 (3H, m), 1.65 (2H, m), 1.86 (1H, m), 1.6 (3H, m), 1.5-1.27 (9H, m), 0.92 (6H, m).

Example 17

Compound 18: To a solution of compound 17 (148 mg, 0.240 mmol) in 4 mL of Methanol was added (1,2,3,4-Tetrahydro-isoquinolin-6-ylmethyl)-phosphonic acid diethyl ester (70.0 mg, 0.240 mmol), acetic acid (43.0 μ L, 0.720 mmol). The reaction mixture was stirred for 3 minutes, followed by addition of Sodium Cyanoborohydride (75.3 mg, 1.20 mmol). The reaction mixture was stirred at 25°C for 14 hours. The reaction mixture was diluted with EtOAc and washed with H₂O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH₂Cl₂/Isopropanol= 100/5) to give 59 mg of TES protected intermediate.

83 μ L of 48% HF solution was added to acetonitrile (4 mL) to prepare the 2% HF solution. The above 2% HF solution was added to TES protected intermediate (47 mg, 0.053 mmol) and the reaction mixture was stirred for 2 hours. The solvent was concentrated and the residue was diluted with EtOAc, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH₂Cl₂/Methanol= 100/10) to give 35.2 mg of compound 18: ¹H NMR (CDCl₃) δ 7.73 (2H, d, J=8.9 Hz), 7.05 (2H, m), 6.89 (2H, m), 6.76 (1H, m), 5.75 (1H, m), 5.67 (1H, m), 5.3 (2H, m), 4.2-3.6 (12 H, m), 3.4-2.4 (11 H, m), 2.1-1.8 (6H, m), 1.4-1.28 (8 H, m), 0.92(6H, m).

Scheme 7

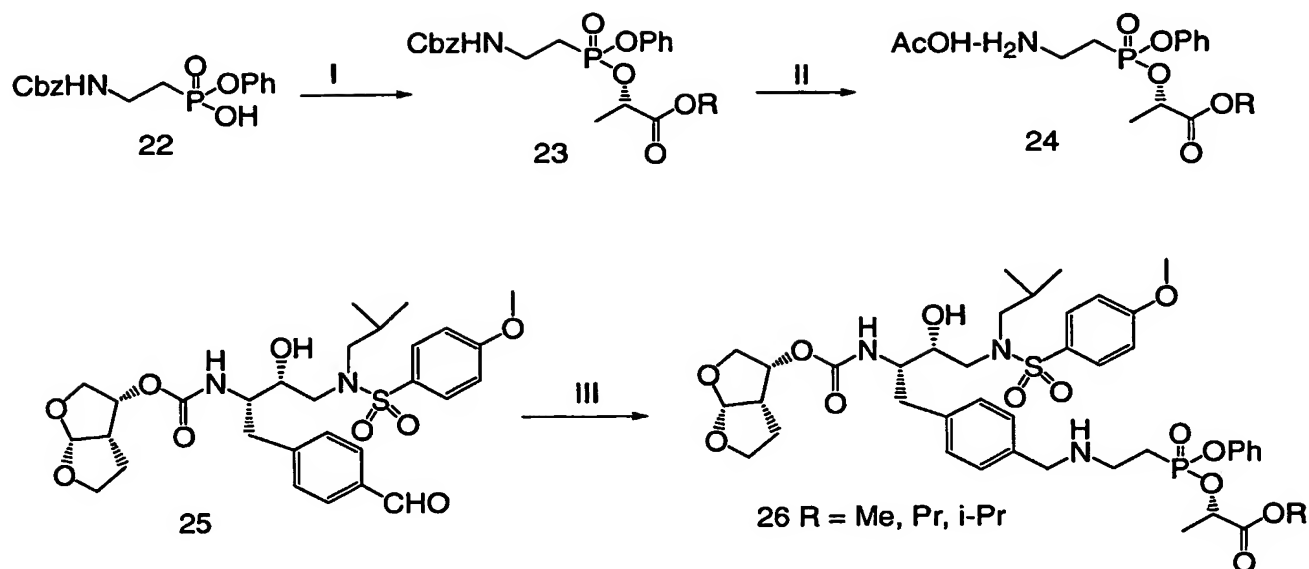


I. Isopropanol/Benzotriazol-1-yloxytripyrrolidinophosphonium
 hexafluorophosphate/ DIPEA/DMF;
 II. H_2 /10%Pd-C/EtOAc-EtOH;
 III. RNH_2 /Aldrithiol-2/ PPh_3 / iPr_2NEt /pyridine

Compound 19 is prepared following the procedure for compound 2 by using monoacid 1.

- 5 Compound 20 is made following a hydrogenation of compound 19. Mono acid 20 reacts with corresponding amino esters in the presence of Aldrithiol-2 and triphenylphosphine to form compound 21.

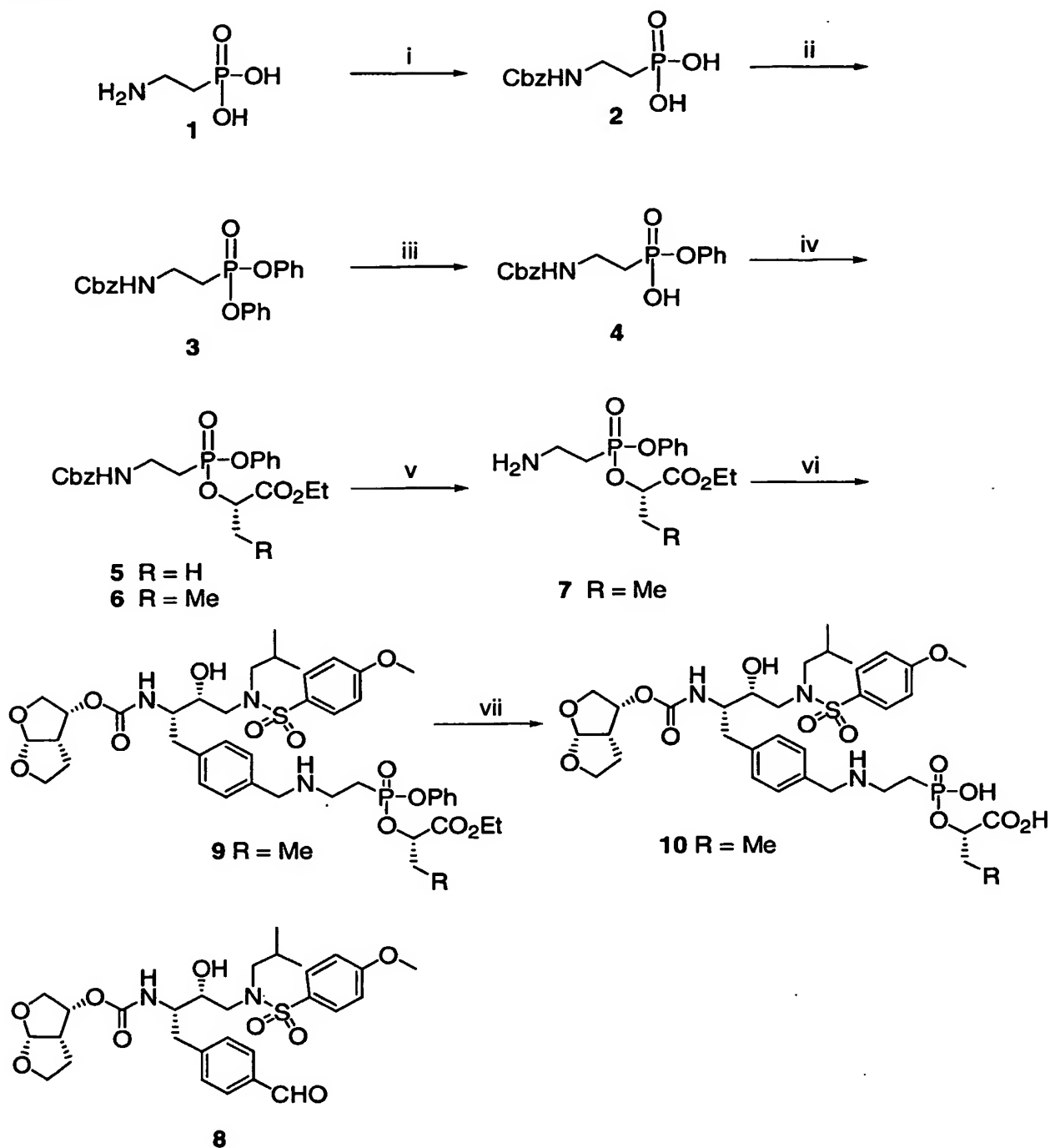
Scheme 8



I. a. SOCl₂/60 °C; b. Alkyl (s)-lactate/Et₃N; II. H₂/10%Pd-C/EtOAc-HOAc;
 III. a. compound 25/MgSO₄; b. HOAc/NaBH₃CN

Monoacid 22 is treated with thionyl chloride at 60°C to form monochloridate, which reacts with corresponding alkyl (s)lactate to generate monolactate 23. Monolactate 23 is hydrogenated with 10%Pd-C in the presence of acetic acid to form amine 24. Aldehyde 25 reacts with amine 24 in the presence of MgSO₄ to form the intermediate imine, which is reduced with sodium cyanoborohydride to afford compound 26.

Scheme 1



Reagents and conditions: i. CbzCl, NaOH, toluene/H₂O, 100%; ii. a. SOCl₂, DMF, toluene, 65°C; b. PhOH, Et₃N, CH₂Cl₂, 71%; iii. aq. NaOH, CH₃CN, 79%; iv. a. SOCl₂, DMF, toluene, 65°C; b. ethyl lactate, Et₃N, CH₂Cl₂, (5) 85%; 2-hydroxy butyric acid ethyl ester, Et₃N, CH₂Cl₂, (6) 75%; v. H₂, AcOH, 10% Pd/C, EtOH, 94%; vi. a. 7 + 8, 1,2-DCE, MgSO₄; b. NaBH₃CN, AcOH, 50%; vii. pig liver esterase, 20% DMSO/PBS, 40°C, 25%.

Example 1

Compound 2: A 3L, 3-neck flask was equipped with a mechanical stirrer and addition funnel and charged with 2-aminoethyl phosphonic acid (60.0g, 480 mmol). 2N Sodium hydroxide
5 (480 mL, 960 mmol) was added and flask cooled to 0°C. Benzyl chloroformate (102.4 g, 600 mmol) in toluene (160mL) was added dropwise with vigorous stirring. The reaction mixture was stirred at 0°C for 30 minutes, then at room temperature for 4 h. 2N sodium hydroxide (240 mL, 480 mmol) was added, followed by benzyl chloroformate (20.5 g, 120 mmol) and the reaction mixture was vigorously stirred for 12 h. The reaction mixture was washed with
10 diethyl ether (3x). The aqueous layer was acidified to pH 2 with concentrated HCl to give a white precipitate. Ethyl acetate was added to the mixture and concentrated HCl (80 mL, 960 mmol) was added. The aqueous layer was extracted with ethyl acetate and combined organic layer was dried (MgSO₄) and concentrated to give a waxy, white solid (124 g, 479 mmol, 100%). ¹H NMR (300 MHz, CD₃OD): δ 7.45-7.30 (m, 5 H, Ar), 5.06 (d, *J* = 14.7 Hz, 2 H, CH₂Ph), 3.44-3.31 (m, 2 H, NCH₂CH₂), 2.03-1.91 (m, 2 H, CH₂CH₂P); ³¹P NMR (121 MHz, CD₃OD): δ 26.3.

Example 2

Compound 3: To a mixture of compound 2 (50.0 g, 193 mmol) in toluene (1.0 L) was added
20 DMF (1.0 mL) followed by thionyl chloride (56 mL, 768 mmol). The reaction mixture was heated at 65°C for 3-4 h under a stream of argon. The reaction mixture was cooled to room temperature and concentrated. Residual solvent was removed under high vacuum for 1 h. The residue was dissolved in CH₂Cl₂ (1.0 L) and cooled to 0°C. Triethylamine (161 mL, 1158 mmol) was added, followed by phenol (54.5 g, 579 mmol). The reaction mixture was
25 warmed to room temperature overnight, then washed with 1.0N HCl, saturated NaHCO₃ solution, brine and dried (MgSO₄). Concentrated and purified (silica gel, 1:1 EtOAc/Hex) to give a pale yellow solid (56 g, 136 mmol, 71%). ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.10 (m, 15 H, Ar), 5.53 (br s, 1 H, NH), 5.11 (br s, 2 H, CH₂Ph), 3.72-3.60 (m, 2 H, NCH₂CH₂), 2.49-2.30 (m, 2 H, CH₂CH₂P); ³¹P NMR (121 MHz, CDCl₃): δ 22.9.

Example 3

Compound 4: To a solution of compound 3 (64 g, 155.6 mmol) in acetonitrile (500 mL) at 0°C was added 2.0M sodium hydroxide. The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 2.5 h. The reaction mixture was concentrated to 100 mL and diluted with H₂O (500 mL). The aqueous solution was washed with EtOAc (3 x 300 mL).
5 The aqueous layer was acidified to pH 1 with concentrated HCl, producing a white precipitated. The mixture was extracted with EtOAc (4 x 300 mL) and combined organic layer was washed with brine and dried (MgSO₄). Concentration gave a solid, which was recrystallized from hot EtOAc (450 mL) to give a white solid (41.04 g, 122 mmol, 79%). ¹H NMR (300 MHz, CD₃OD): δ 7.45-7.10 (m, 10 H, Ar), 5.09 (s, 2 H, CH₂Ph), 3.53-3.30 (m, 2
10 H, NCH₂CH₂), 2.25-2.10 (m, 2 H, CH₂CH₂P); ³¹P NMR (121 MHz, CD₃OD): δ 24.5.

Example 4

Compound 5: To a mixture of compound 4 (28 g, 83 mmol) in toluene (500 mL) was added DMF (1.0 mL), followed by thionyl chloride (36.4 mL, 499 mmol). The mixture was heated
15 at 65°C for 2 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous CH₂Cl₂ (350 mL) and cooled to 0°C. Triethylamine (45.3 mL, 332 mmol) was added slowly, followed by the dropwise addition of ethyl lactate (18.8 mL, 166 mmol). The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature overnight. The reaction mixture was
20 diluted with CH₂Cl₂ and washed with 1 N HCl, saturated NaHCO₃ solution, brine and dried (MgSO₄). Concentration and purification (silica gel, 1:5 to 1:0 EtOAc/Hex) gave a pale yellow oil (30.7 g, 71 mmol, 85%) as a mixture of diastereomers which were separated by HPLC (Dynamax reverse phase C-18 column, 60% acetonitrile/H₂O). More polar diastereomer: ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.10 (m, 10 H, Ar), 5.65 (s, 1 H, NH),
25 5.12 (s, 2 H, CH₂Ph), 5.10-5.00 (m, 1 H, OCHC) 4.17 (q, *J* = 6.9 Hz, 2 H, OCH₂CH₃), 3.62 (dt, *J*₁ = 20.4 Hz, *J*₂ = 6.0 Hz, 2 H, NCH₂CH₂), 2.25 (dt, *J*₁ = 18.0 Hz, *J*₂ = 6.0 Hz, 2 H, CH₂CH₂P), 1.60 (dd, *J*₁ = *J*₂ = 6.9 Hz, 3 H, CHCH₃), 1.23 (t, *J* = 6.9 Hz, 3 H, OCH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): δ 26.2. Less polar diastereomer: ¹H NMR (300 MHz, CDCl₃): δ
30 7.40-7.10 (m, 10 H, Ar), 5.87 (s, 1 H, NH), 5.13 (s, 2 H, CH₂Ph), 5.10-5.00 (dq, *J*₁ = *J*₂ = 6.9 Hz, 1 H, OCHC) 4.22 (q, *J* = 7.2 Hz, 2 H, OCH₂CH₃), 3.68 (dt, *J*₁ = 21.6 Hz, *J*₂ = 6.9 Hz, 2 H, NCH₂CH₂), 2.40-2.20 (m, 2 H, CH₂CH₂P), 1.49 (dd, *J*₁ = 70.2 Hz, *J*₂ = 6.9 Hz, 3 H, CHCH₃), 1.28 (t, *J* = 6.9 Hz, 3 H, OCH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): δ 28.3.

Example 5

Compound 6: 2-Hydroxy-butyric acid ethyl ester was prepared as follows: To a solution of L-2-aminobutyric acid (100g, 970 mmol) in 1.0 N H₂SO₄ (2 L) at 0°C was added NaNO₂ (111 g, 1610 mmol) in H₂O (400 mL) over 2 h. The reaction mixture was stirred at room temperature for 18h. Reaction mixture was extracted with EtOAc (4x) and combined organic layer was dried (MgSO₄) and concentrated to give a yellow solid (41.5 g). This solid was dissolved in absolute ethanol (500 mL) and concentrated HCl (3.27 mL, 39.9 mmol) was added. Reaction mixture was heated to 80°C. After 24 h, concentrated HCl (3 mL) was added and reaction continued for 24 h. Reaction mixture was concentrated and product was distilled to give a colorless oil (31 g, 235 mmol, 59%).

To a mixture of compound 4 (0.22 g, 0.63 mmol) in anhydrous acetonitrile (3.0 mL) was added thionyl chloride (0.184 mL, 2.52 mmol). The mixture was heated at 65°C for 1.5 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous CH₂Cl₂ (3.3 mL) and cooled to 0°C. Triethylamine (0.26 mL, 1.89 mmol) was added slowly, followed by the dropwise addition of 2-hydroxy-butyric acid ethyl ester (0.167 mL, 1.26 mmol). The reaction mixture was stirred at 0°C for 5 min, then warmed to room temperature overnight. The reaction mixture was concentrated, dissolved in EtOAc and washed with 1.0 N HCl, saturated NaHCO₃ solution, brine and dried (MgSO₄). Concentration and purification (silica gel, 3:2 EtOAc/Hex) gave a pale yellow oil (0.21 g, 0.47 mmol, 75%). For major diastereomer, ¹H NMR (300 MHz, CDCl₃): δ 7.35-7.10 (m, 10 H, Ar), 5.91 (s, 1 H, NH), 5.12 (s, 2 H, CH₂Ph), 4.94-4.83 (m, 1 H, OCHC), 4.27-4.12 (m, 2 H, OCH₂CH₃), 3.80-3.50 (m, 2 H, NCH₂CH₂), 2.39-2.19 (m, 2 H, CH₂CH₂P), 1.82-1.71 (m, 2 H, CHCH₂CH₃), 1.30-1.195 (m, 3 H, OCH₂CH₃), 0.81 (t, *J* = 7.5 Hz, 3 H, CHCH₂CH₃); ³¹P NMR (120 MHz, CDCl₃): δ 28.3. For minor diastereomer, ¹H NMR (300 MHz, CDCl₃): δ 7.35-7.10 (m, 10 H, Ar), 5.74 (s, 1 H, NH), 5.11 (s, 2 H, CH₂Ph), 4.98-4.94 (m, 1 H, OCHC), 4.27-4.12 (m, 2 H, OCH₂CH₃), 3.80-3.50 (m, 2 H, NCH₂CH₂), 2.39-2.19 (m, 2 H, CH₂CH₂P), 1.98-1.82 (m, 2 H, CHCH₂CH₃), 1.30-1.195 (m, 3 H, OCH₂CH₃), 1.00 (t, *J* = 7.5 Hz, 3 H, CHCH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): δ 26.2.

Example 6

Compound 7: A mixture of compound 6, (0.53 g, 1.18 mmol) acetic acid (0.135 mL, 2.36 mmol) and 10% palladium on activated carbon (0.08 g) in absolute ethanol (12 mL) was stirred under a hydrogen atmosphere (1 atm) for 3 h. Reaction mixture was filtered through Celite, concentrated, and resubjected to identical reaction conditions. After 2 h, Celite was added to the reaction mixture and mixture was stirred for 2 min, then filtered through a pad of Celite and concentrated. Dried under high vacuum to give the diastomeric acetate salt as a oil (0.42 g, 1.11 mmol, 94%). ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.10 (m, 5 H, Ar), 5.00-4.80 (m, 1 H, OCHC), 4.28-4.10 (m, 2 H, OCH₂CH₂), 3.32-3.14 (m, 2 H, NCH₂CH₂), 2.45-2.22 (m, 2 H, CH₂CH₂P), 1.97 (s, 3 H, Ac), 1.97-1.70 (m, 2 H, CHCH₂CH₃), 1.30-1.18 (m, 3 H, OCH₂CH₃), 1.00 (t, *J* = 7.5 Hz, 1 H, CHCH₂CH₃), 0.80 (t, *J* = 7.5 Hz, 2 H, CHCH₂CH₃); ³¹P NMR (121 MHz, CDCl₃): δ 27.6 (major, 1.85), 26.0 (minor, 1.01).

Example 7

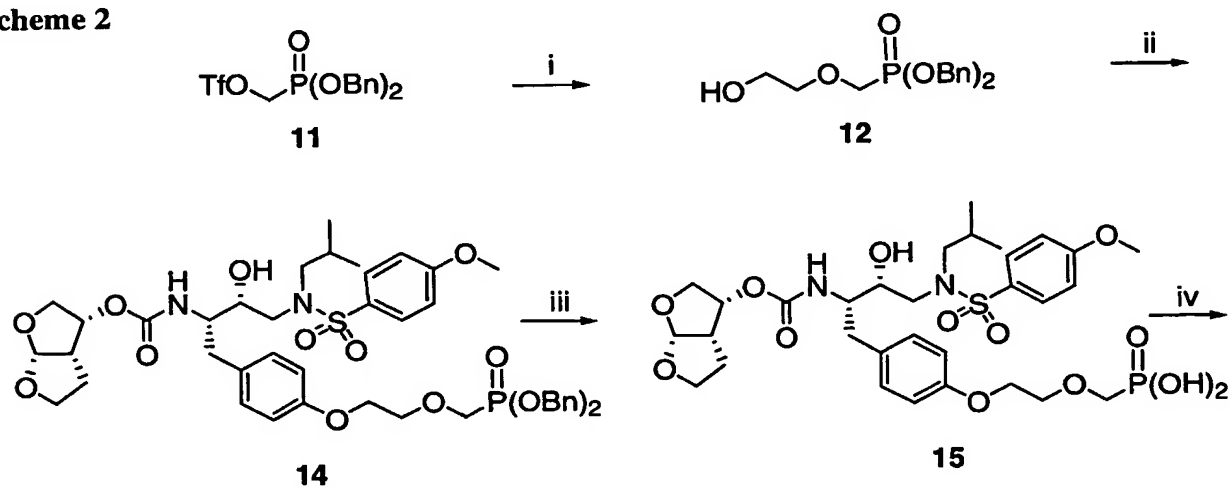
Compound 9: A solution of aldehyde 8 (0.596 g, 1.01 mmol) and compound 7 (0.42 g, 1.11 mmol) were stirred together in 1,2-dichloroethane (4.0 mL) in the presence of MgSO₄ for 3 h. Acetic acid (0.231 mL, 4.04 mmol) and sodium cyanoborohydride (0.127 g, 2.02 mmol) were added and reaction mixture was stirred for 50 min at room temperature. Reaction mixture was quenched with saturated NaHCO₃ solution, diluted with EtOAc, and vigorously stirred for 5 min. Brine was added and extracted with EtOAc (2x). Combined organic layer was dried (MgSO₄) concentrated and purified (silica gel, EtOAc, then 10% EtOH/EtOAc) to give a colorless foam. Acetonitrile (4 mL) and trifluoroacetic acid (0.06 mL) were added and concentrated to a volume of 1 mL. H₂O (10 mL) was added and lyophilized to give the TFA salt as a white powder (0.51 g, 0.508 mmol, 50%). ¹H NMR (300 MHz, CD₃CN): δ 7.79 (d, *J* = 8.4 Hz, 2 H, (SO₂C(CH)₂), 7.43-7.20 (m, 9 H, Ar), 7.10 (d, *J* = 8.4 Hz, 2 H, (CH)₂COCH₃), 5.85 (d, *J* = 8.4 Hz, 1 H, NH), 5.55 (d, *J* = 4.5 Hz, 1 H, OCHO), 5.00-4.75 (m, 2 H, CH₂CHOC(O), POCHC), 4.39-4.05 (m, 2 H, PhCH₂N, OCH₂CH₃), 3.89 (s, 3 H, OCH₃), 3.88-3.30 (m, 9H), 3.15-2.84 (m, 5 H), 2.65-2.42 (m, 3 H), 2.10-1.68 (m, 5 H), 1.65-1.15 (m, 5 H), 1.05-0.79 (m, 9 H); ³¹P NMR (121 MHz, CD₃CN): δ 24.8 (major, 1.85), 23.1 (minor, 1.01).

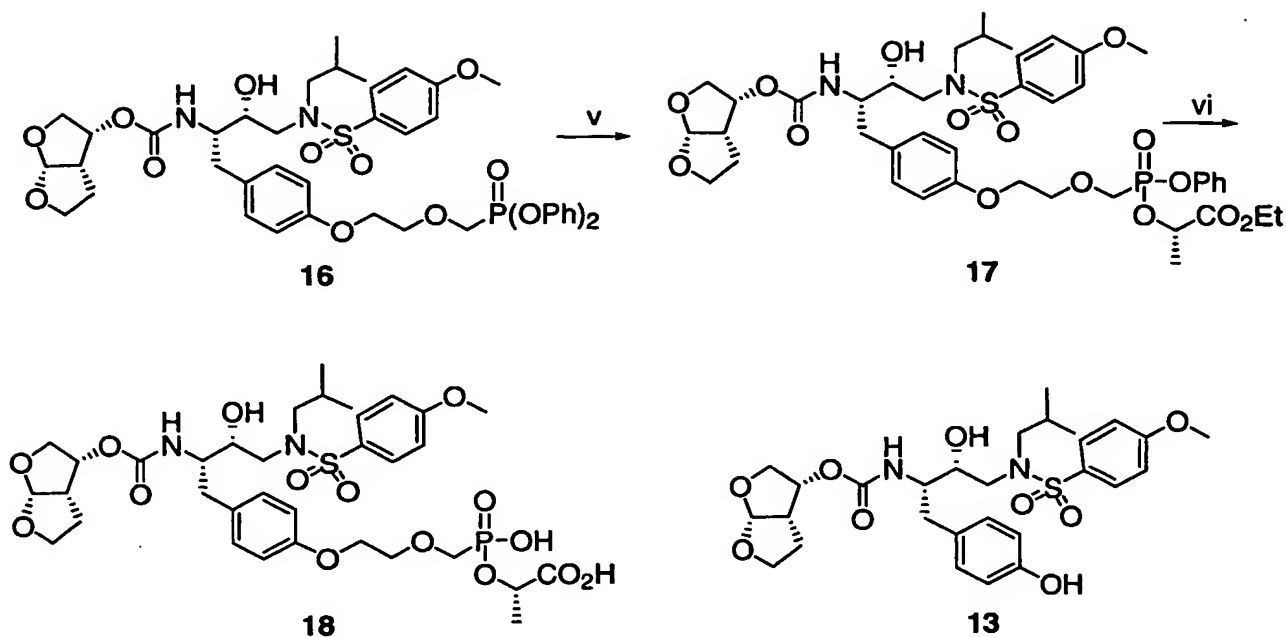
Example 8

Compound 10: Compound 9 (0.041 g, 0.041 mmol) was dissolved in DMSO (1.9 mL) and to this solution was added phosphate buffered saline, pH 7.4 (10 mL) and pig liver esterase

(Sigma, 0.2 mL). Reaction mixture was stirred for 24 h at 40°C. After 24 h, additional esterase (0.2 mL) was added and reaction was continued for 24 h. Reaction mixture was concentrated, resuspended in methanol and filtered. Filtrate was concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (8 mg, 0.010 mmol, 25%). ¹H NMR (500 MHz, CD₃OD): δ 7.78 (d, *J* = 8.9 Hz, 2 H, (SO₂C(CH)₂), 7.43-7.35 (m, 4 H, Ar), 7.11 (d, *J* = 8.9 Hz, 2 H, (CH)₂COCH₃), 5.62 (d, *J* = 5.2 Hz, 1 H, OCHO), 4.96-4.77 (m, 2 H, CH₂CHOC(O), POCHC), 4.21 (br s, 2 H, PhCH₂N), 3.97-3.70 (m, 6 H), 3.90 (s, 3 H, OCH₃), 3.50-3.30 (m, 3 H), 3.26-3.02 (m, 2 H), 2.94-2.58 (m, 4 H), 2.09-1.78 (m, 5 H), 1.63-1.52 (m, 2 H), 1.05-0.97 (m, 3 H); 0.94 (d, *J* = 6.7 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); ³¹P NMR (121 MHz, CD₃OD): δ 20.8.

Scheme 2





Reagents and conditions: i. ethylene glycol, $\text{Mg}(\text{OtBu})_2$, DMF, 48%; ii. a. Tf_2O , 2,6-lutidine, CH_2Cl_2 , -78°C ; b. 13, CsCO_3 , CH_3CN , 0°C to room temperature, 65%; iii. H_2 , Pd/C, EtOH, 107%; iv. DCC, PhOH, pyr, 70°C , 31%; v. a. NaOH, CH_3CN , 0°C ; b. DCC, ethyl lactate, pyr, 70°C , 52%; vi. CH_3CN , DMSO, PBS, porcine liver esterase, 38°C , 69%.

Example 9

- 5 Compound 12: To a solution of compound 11 (4.10 g, 9.66 mmol) and anhydrous ethylene glycol (5.39 mL, 96.6 mmol) in anhydrous DMF (30 mL) at 0°C was added powdered magnesium *tert*-butoxide (2.05 g, 12.02 mmol). The reaction mixture was stirred at 0°C for 1.5 h, then concentrated. The residue was partitioned between EtOAc and H_2O and washed with 1 N HCl, saturated NaHCO_3 solution, and brine. Organic layer dried (MgSO_4),
- 10 concentrated and purified (silica gel, 4% MeOH/ CH_2Cl_2) to give a colorless oil (1.55 g, 48%). ^1H NMR (300 MHz, CDCl_3): δ 7.37 (s, 10 H, Ar), 5.40-5.05 (m, 4 H, CH_2Ph), 3.84 (d, $J = 8.1$ Hz, 2 H, PCH_2O), 3.70-3.60 (m, 4 H, $\text{OCH}_2\text{CH}_2\text{O}$, $\text{OCH}_2\text{CH}_2\text{O}$); ^{31}P NMR (121 MHz, CDCl_3): δ 22.7.

15 Example 10

Compound 14: To a solution of compound 12 (0.75 g, 2.23 mmol) and 2,6-lutidine (0.78 mL, 6.69 mmol) in CH_2Cl_2 (20 mL) at -78°C was added trifluoromethanesulfonic anhydride (0.45

mL, 2.68 mmol). The reaction mixture was stirred at -78°C for 40 min, then diluted with CH_2Cl_2 and washed with 1 N HCl, saturated NaHCO_3 and dried (MgSO_4). Concentration gave a yellow oil that was dissolved in anhydrous acetonitrile (20 mL). Phenol **13** (1.00 g, 1.73 mmol) was added to the solution, which was cooled to 0°C . Cesium carbonate (0.619 g, 1.90 mmol) was added and reaction mixture was stirred at 0°C for 2 h, then at room temperature for 1.5 h. Additional cesium carbonate (0.200 g, 0.61 mmol) was added and reaction was continued for 1.5 h, then filtered. Concentration of the filtrate and purification (silica gel, 3% MeOH/ CH_2Cl_2) gave a yellow gum (1.005 g, 65%). ^1H NMR (300 MHz, CDCl_3): δ 7.71 (d, $J = 8.7$ Hz, 2 H, $\text{SO}_2\text{C}(\text{CH})_2$), 7.34 (s, 10 H, PhCH_2O), 7.11 (d, $J = 8.1$ Hz, 2 H, $\text{CH}_2\text{C}(\text{CH})_2(\text{CH})_2$), 6.98 (d, $J = 8.7$ Hz, 2 H, $(\text{CH})_2\text{COCH}_3$), 6.78 (d, $J = 8.7$ Hz, 2 H, $(\text{CH})_2\text{COCH}_2$), 5.62 (d, $J = 5.4$ Hz, 1 H, OCHO), 5.16-4.97 (m, 6 H), 4.05-3.65 (m, 12 H), 3.86 (s, 3 H, OCH_3), 3.19-2.66 (m, 7 H), 1.95-1.46 (m, 3 H), 0.92 (d, $J = 6.6$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$), 0.88 (d, $J = 6.6$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$); ^{31}P NMR (121 MHz, CDCl_3): δ 21.9.

15 Example 11

Compound 15: A mixture of compound **14** (0.410 g, 0.457 mmol) and 10% palladium on carbon (0.066 g) in ethanol (5.0 mL) was stirred under a hydrogen atmosphere (1 atm) for 16 h. Celite was added and the mixture was stirred for 5 min, then filtered through Celite and concentrated to give a foam (0.350 g, 107%). ^1H NMR (300 MHz, CD_3OD): δ 7.76 (d, $J = 8.7$ Hz, 2 H, $\text{SO}_2\text{C}(\text{CH})_2$), 7.15 (d, $J = 8.4$ Hz, 2 H, $\text{CH}_2\text{C}(\text{CH})_2(\text{CH})_2$), 7.08 (d, $J = 8.4$ Hz, 2 H, $(\text{CH})_2\text{COCH}_3$), 6.82 (d, $J = 8.4$ Hz, 2 H, $(\text{CH})_2\text{COCH}_2$), 5.59 (d, $J = 5.4$ Hz, 1 H, OCHO), 5.16-4.97 (masked by CD_3OH , 1 H), 4.09-4.02 (m, 2 H), 3.99-3.82 (m, 10 H), 3.88 (s, 3 H, OCH_3), 3.52-3.32 (m, 1 H), 3.21-2.75 (m, 5 H), 2.55-2.40 (m, 1 H), 2.10-1.95 (m, 1 H), 1.75-1.25 (m, 2 H), 0.93 (d, $J = 6.3$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$), 0.88 (d, $J = 6.6$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$); ^{31}P NMR (121 MHz, CD_3OD): δ 19.5.

Example 12

Compound 16: Compound **15** (0.350 g, 0.488 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL), each time filling with N_2 . Residue was dissolved in anhydrous pyridine (2.5 mL) and phenol (0.459 g, 4.88 mmol) was added. This solution was heated to 70°C , then 1,3-dicyclohexylcarbodiimide (0.403 g, 1.93 mmol) was added and reaction mixture was heated at 70°C for 7 h. Reaction mixture was concentrated, coevaporated with toluene and

residue obtained was diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 2% MeOH/CH₂Cl₂, then another column 75% EtOAc/Hex) to give a clear oil (0.1324 g, 31%).
¹H NMR (300 MHz, CDCl₃): δ 7.71 (d, *J* = 8.7 Hz, 2 H, SO₂C(CH)₂), 7.41-7.18 (m, 10 H, Ar), 7.14 (d, *J* = 8.4 Hz, 2 H, CH₂C(CH)₂(CH)₂), 6.99 (d, *J* = 9.0 Hz, 2 H, (CH)₂COCH₃), 6.83 (d, *J* = 8.4 Hz, 2 H, (CH)₂COCH₂), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 2 H), 4.32-3.62 (m, 12 H), 3.87 (s, 3 H, OCH₃), 3.22-2.73 (m, 7 H), 1.95-1.75 (m, 3 H), 0.93 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂); ³¹P NMR (121 MHz, CDCl₃): δ 14.3.

10

Example 13

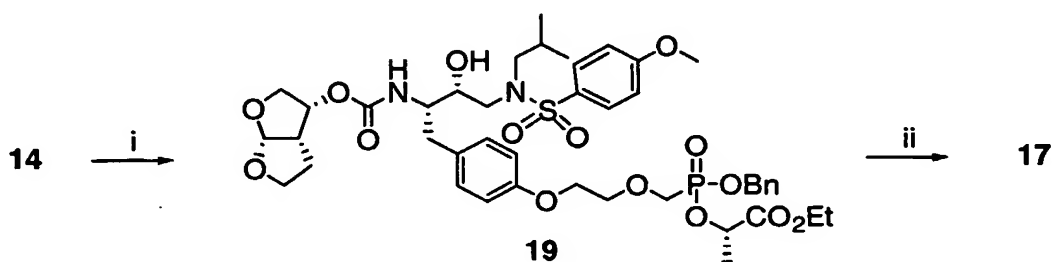
Compound 17: To a solution of compound 16 (0.132 g, 0.152 mmol) in acetonitrile (1.5 mL) at 0°C was added 1.0 M NaOH (0.38 mL, 0.381 mmol). Reaction mixture was stirred for 2 h at 0°C, then Dowex 50 (H+) resin was added until pH = 1. The resin was removed by
15 filtration and the filtrate was concentrated and washed with EtOAc/Hex (1:2, 25 mL), then dried under high vacuum to give a clear film (0.103 g, 85%). This film was coevaporated with anhydrous pyridine (3 x 5 mL), filling with N₂. The residue was dissolved in anhydrous pyridine (1 mL) and ethyl lactate (0.15 mL, 1.30 mmol) was added and reaction mixture was heated at 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (0.107 g, 0.520 mmol) was added
20 and reaction mixture was stirred at 70°C for 2.5 h. Additional 1,3-dicyclohexylcarbodiimide (0.055 g, 0.270 mmol) was added and reaction continued for another 1.5 h. Reaction mixture was concentrated and coevaporated with toluene and diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 80 to 100% EtOAc/Hex) to give a white foam (0.0607 g, 52%).
25 ¹H NMR (300 MHz, CDCl₃): δ 7.71 (d, *J* = 8.7 Hz, 2 H, SO₂C(CH)₂), 7.39-7.16 (m, 5 H, Ar), 7.13 (d, *J* = 8.1 Hz, 2 H, CH₂C(CH)₂(CH)₂), 6.99 (d, *J* = 9.0 Hz, 2 H, (CH)₂COCH₃), 6.82 (d, *J* = 8.4 Hz, 2 H, (CH)₂COCH₂), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 3 H), 4.35-3.65 (m, 14 H), 3.87 (s, 3 H, OCH₃), 3.22-2.73 (m, 7 H), 1.95-1.80 (m, 3 H), 1.59 (d, *J* = 6.9 Hz, 1.5 H, CCHCH₃), 1.47 (d, *J* = 7.2 Hz, 1.5 H, CCHCH₃), 1.37-1.18 (m, 3 H), 0.92 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂); ³¹P NMR (121 MHz, CDCl₃): δ 19.2, 17.2.
30

Example 14

Compound 18: Compound **17** (11.5 mg, 0.013 mmol) was dissolved in DMSO (0.14 mL) and acetonitrile (0.29 mL). PBS (pH 7.4, 1.43 mL) was added slowly with stirring. Porcine liver esterase (Sigma, 0.1 mL) was added and reaction mixture was gently stirred at 38°C.

- 5 After 24 h, additional porcine liver esterase (0.1 mL) and DMSO (0.14 mL) were added and reaction mixture stirred for 48 h at 38°C. Reaction mixture concentrated and methanol was added to precipitate the enzyme. The mixture was filtered, concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (7.1 mg, 69%). ¹H NMR (300 MHz, CD₃OD): δ 7.76 (d, *J* = 8.7 Hz, 2 H, SO₂C(CH)₂), 7.15 (d, *J* = 8.4 Hz, 2 H, CH₂C(CH)₂(CH)₂), 7.08 (d, *J* = 9.0 Hz, 2 H, (CH)₂COCH₃), 6.83 (d, *J* = 8.7 Hz, 2 H, (CH)₂COCH₂), 5.59 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.90 (masked by CD₃OH, 2 H), 4.19-3.65 (m, 12 H), 3.88 (s, 3 H, OCH₃), 3.50-3.27 (m, 1 H), 3.20-2.78 (m, 5 H), 2.55-2.40 (m, 1 H), 2.05-1.90 (m, 1 H), 1.75-1.30 (m, 2 H), 1.53 (d, *J* = 6.6 Hz, 3 H, CCHCH₃), 0.93 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH₃)₂); ³¹P NMR (121 MHz, CD₃OD):
- 10 δ 16.7.
- 15

Alternatively, compound **17** was prepared as described below (Scheme 3).

Scheme 3

Reagents and conditions: i. a. **14**, DABCO, toluene, reflux; b. ethyl lactate, PyBOP, DIPEA, DMF, 59%;
ii. a. H₂, Pd/C, EtOH; b. PhOH, PyBOP, DIPEA, DMF, 35%.

Example 15

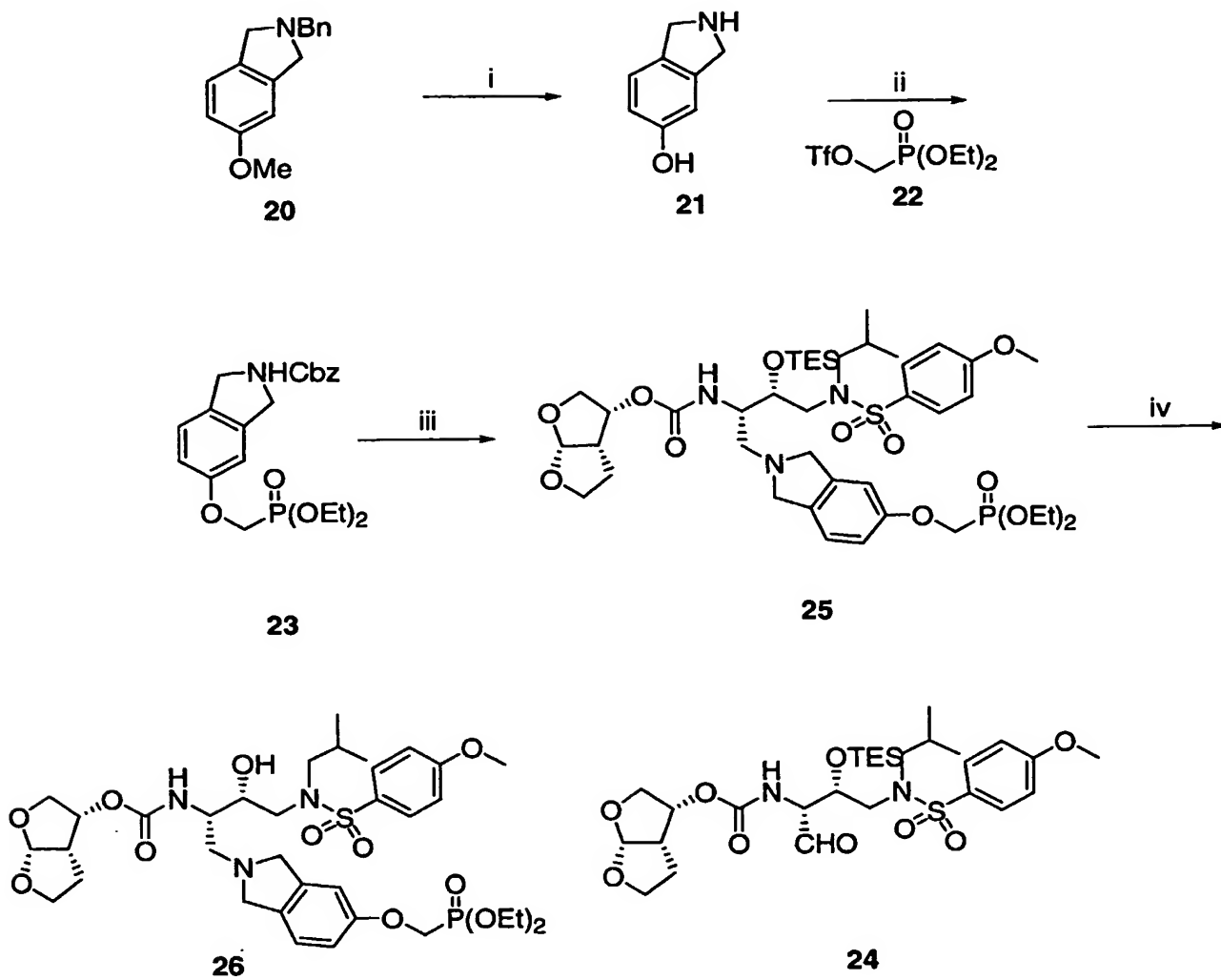
Compound 19: To a solution of compound 14 (0.945 g, 1.05 mmol) in anhydrous toluene (10.0 mL) was added 1,4-diazobicyclo[2.2.2] octane (0.130 g, 1.16 mmol) and reaction mixture was refluxed for 2 h. After cooling to room temperature, reaction mixture was
5 diluted with EtOAc and washed with 1.0 N HCl and dried (MgSO₄). Concentration gave a white foam (0.785 g, 93%). Residue was dissolved in anhydrous DMF (10.0 mL) and to this solution was added ethyl (S)-lactate (0.23 mL, 2.00 mmol) and diisopropylethylamine (0.70 mL, 4.00 mmol), followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (1.041 g, 2.00 mmol). Reaction mixture was stirred for 20 h, then
10 concentrated and residue was dissolved in EtOAc and washed with 1.0 N HCl, saturated NaHCO₃, brine and dried (MgSO₄). Concentration and purification (silica gel, 2 % MeOH/CH₂Cl₂) gave an off-white foam (0.520 g, 59%). ¹H NMR (300 MHz, CDCl₃): δ 7.72 (d, *J* = 7.5 Hz, 2 H, SO₂C(CH)₂), 7.50-7.27 (m, 4 H, Ar), 7.12 (d, *J* = 8.1 Hz, 2 H, CH₂C(CH)₂(CH)₂), 7.00 (d, *J* = 6.6 Hz, 2 H, (CH)₂COCH₃), 6.81 (d, *J* = 8.4 Hz, 2 H, (CH)₂COCH₂), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.37-4.90 (m, 5 H), 4.35-3.65 (m, 14 H), 3.88 (s, 3 H, OCH₃), 3.24-2.70 (m, 7 H), 1.90-1.70 (m, 3 H), 1.54 (d, *J* = 6.9 Hz, 1.5 H, CCHCH₃), 1.47 (d, *J* = 6.9 Hz, 1.5 H, CCHCH₃), 1.37-1.22 (m, 3 H), 0.93 (d, *J* = 6.3 Hz, 3 H, CH(CH₃)₂), 0.89 (d, *J* = 6.0 Hz, 3 H, CH(CH₃)₂); ³¹P NMR (121 MHz, CDCl₃): δ 22.3, 21.2.

20

Example 16

Compound 17: A mixture of compound 19 (0.520 g, 0.573 mmol) and 10% palladium on carbon (0.055 g) in ethanol (10 mL) was stirred under a hydrogen atmosphere (1 atm) for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then mixture was filtered
25 through Celite and concentrated to give a white foam (0.4649 g, 99%). Residue was dissolved in anhydrous DMF (5.0 mL) and to this solution was added phenol (0.097 g, 1.03 mmol), diisopropylethylamine (0.36 mL, 2.06 mmol) followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (0.536 g, 1.03 mmol). Reaction mixture was stirred for 20 h, then concentrated and residue was dissolved in EtOAc and
30 washed with 1 N HCl, H₂O, sat. NaHCO₃, brine and dried (MgSO₄). Concentration and purification (silica gel, 2 % MeOH/CH₂Cl₂) gave a white foam (0.180 g, 35%).

Scheme 4



Reagents and conditions: i. a. 48% HBr, 120°C, 65%; b. H₂, Pd(OH)₂, EtOH, 100%;
 ii. CbzCl, NaOH, toluene/H₂O, 0°C to rt, 43%; b. **22**, CsCO₃, CH₃CN, 99%;
 iii. a. H₂, Pd/C, AcOH, EtOAc/EtOH, 95%; b. **24**, NaBH(OAc)₃, 1,2-DCE, 21%;
 iv. 4% HF/CH₃CN, 62%.

Example 17

- 5 Compound 21: Compound **20** (11.5 g, 48.1 mmol) in 48% HBr (150 mL) was heated at 120°C for 4 h, then cooled to room temperature and diluted with EtOAc. Mixture was neutralized with saturated NaHCO₃ solution and solid NaHCO₃ and extracted with EtOAc containing MeOH. Organic layer dried (MgSO₄), concentrated, and purified (silica gel, 1:2

EtOAc/Hex with 1% MeOH) to give a brown solid (7.0 g, 65%). The resulting compound (7.0 g, 31.1 mmol) and 10% palladium hydroxide (2.1 g) in EtOH (310 mL) was stirred under a hydrogen atmosphere for 1 d, then filtered through Celite and concentrated to give an off-white solid (4.42 g, 100%). ¹H NMR (300 MHz, CDCl₃): δ 7.01 (d, *J* = 7.8 Hz, 1 H, Ar), 6.64 (s, 1 H, Ar), 6.61 (d, *J* = 8.1 Hz, 2 H, Ar), 4.07 (s, 2 H, ArCH₂N), 4.05 (s, 2 H, ArCH₂N).

Example 18

Compound 22: To a solution of compound 21 (4.42 g, 32.7 mmol) in 1.0 M NaOH (98 mL, 98.25 mmol) at 0°C was added dropwise benzyl chloroformate (7.00 mL, 49.13 mmol) in toluene (7 mL). After addition was complete, reaction mixture was stirred overnight at room temperature. Reaction mixture was diluted with EtOAc and extracted with EtOAc (3x). Combined organic layer was dried (MgSO₄), concentrated and purified (silica gel, 2% MeOH/CH₂Cl₂) to give a white solid (3.786 g, 43%). The resulting compound (0.6546 g, 2.43 mmol) was dissolved in anhydrous acetonitrile (10 mL), and compound 23 (0.782 g, 2.92 mmol) was added, followed by cesium carbonate (1.583 g, 4.86 mmol). Reaction mixture was stirred for 2 h at room temperature, then filtered, concentrated, and purified (3% MeOH/CH₂Cl₂) to give a brownish oil (1.01 g, 99%).

Example 19

Compound 25: To a solution of compound 22 (0.100 g, 0.238 mmol) in EtOAc/EtOH (2 mL, 1:1) was added acetic acid (14 μL, 0.238 mmol) and 10% palladium on carbon (0.020 g) and the mixture was stirred under a hydrogen atmosphere for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then filtered through Celite. Concentration and drying under high vacuum gave a reddish film (0.0777 g, 95%). The resulting amine (0.0777 g, 0.225 mmol) and aldehyde 24 (0.126 g, 0.205 mmol) in 1,2-dichloroethane (1.2 mL) were stirred for 5 min at 0°C, then sodium triacetoxyborohydride (0.0608 g, 0.287 mmol) was added. Reaction mixture was stirred for 1 h at 0°C, then quenched with saturated NaHCO₃ solution and brine. Extracted with EtOAc, the organic layer was dried (MgSO₄), concentrated and purified (silica gel, 2% MeOH/CH₂Cl₂) to give a brown foam (38.7 mg, 21%). ¹H NMR (300 MHz, CDCl₃): δ 7.74 (d, *J* = 8.7 Hz, 2 H, Ar), 7.09 (d, *J* = 8.7 Hz, 1 H, Ar), 7.05-6.72 (m, 4 H, Ar), 5.71 (d, *J* = 5.1 Hz, 1 H), 5.22-5.07 (m, 2 H), 4.22-4.17 (m, 7 H),

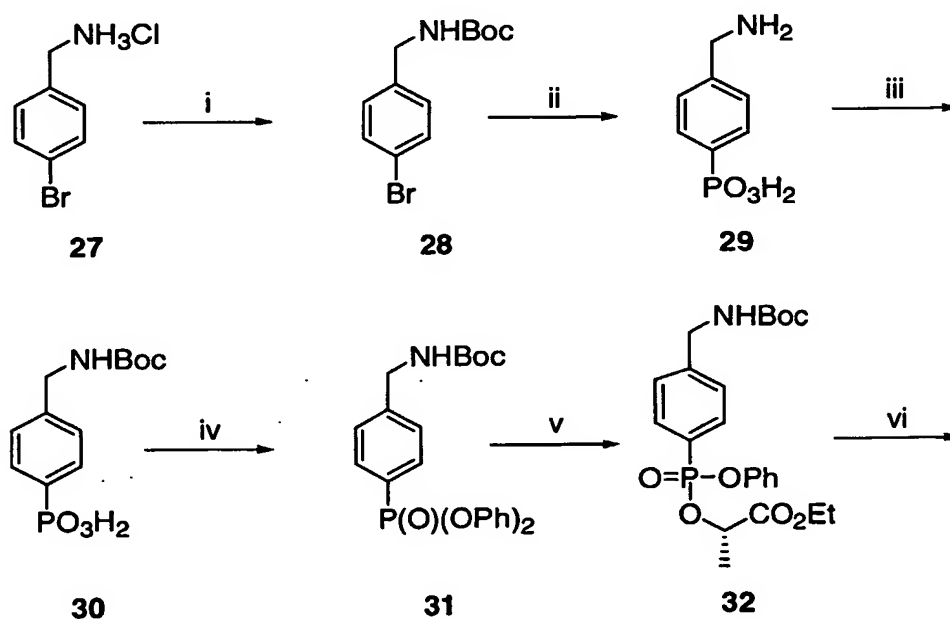
4.16-3.69 (m, 9 H), 3.82 (s, 3 H), 3.25-2.51 (m, 7 H), 2.22-1.70 (m, 3 H), 1.37 (t, $J = 6.9$ Hz, 6 H), 1.10-0.58 (m, 21 H); ^{31}P NMR (121 MHz, CDCl_3): δ 19.5.

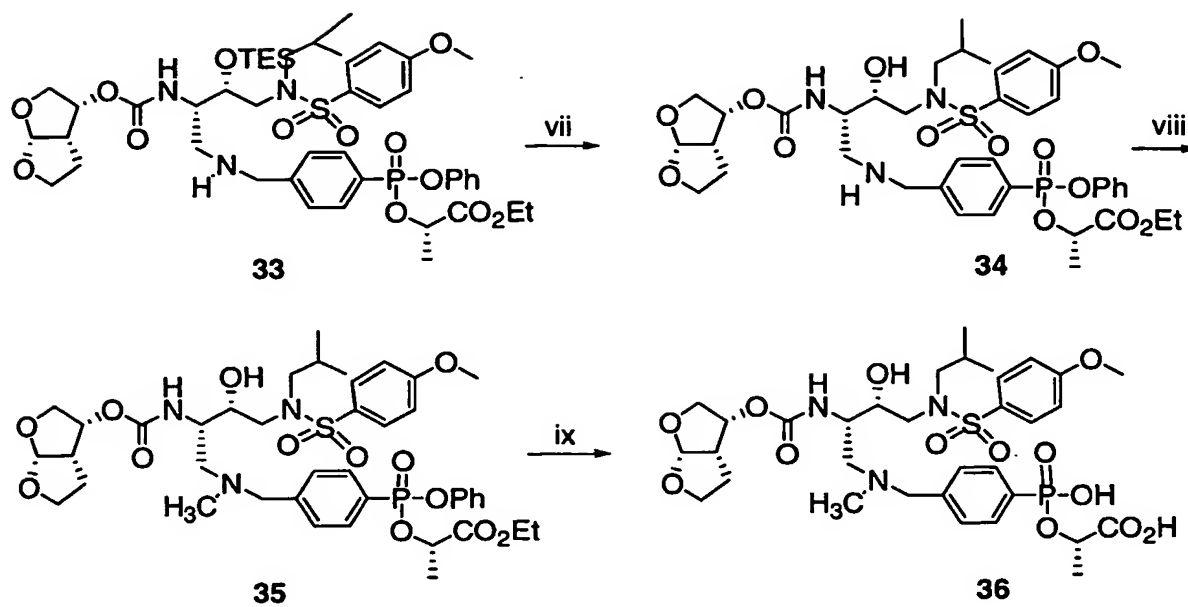
Example 20

- 5 Compound 26: To a solution of compound 25 (38.7 mg, 0.0438 mmol) in acetonitrile (0.5 mL) at 0°C was added 48% HF (0.02 mL). The reaction mixture was stirred at room temperature for 2 h, then quenched with saturated NaHCO_3 solution and extracted with EtOAc. Organic layer was separated, dried (MgSO_4), concentrated and purified (silica gel, 3 to 5% MeOH/ CH_2Cl_2) to give a red film (21.2 mg, 62%). ^1H NMR (300 MHz, CDCl_3): δ
- 10 7.73 (d, $J = 8.7$ Hz, 2 H, Ar), 7.10 (d, $J = 8.7$ Hz, 1 H, Ar), 6.97 (d, $J = 8.70$ Hz, 2 H), 6.90-6.76 (m, 2 H), 5.72 (d, $J = 5.1$ Hz, 1 H), 5.41 (d, $J = 9.0$ Hz, 1 H), 5.15 (q, $J = 6.6$ Hz, 1 H), 4.38-4.17 (m, 7 H), 4.16-3.65 (m, 9 H), 3.87 (s, 3 H), 3.20-2.82 (m, 7 H), 2.75-1.79 (m, 3 H), 1.37 (t, $J = 6.9$ Hz, 6 H), 0.90 (d, $J = 6.6$ Hz, 3 H), 0.88 (d, $J = 6.6$ Hz, 3 H); ^{31}P NMR (121 MHz, CDCl_3): δ 19.3.

15

Scheme 5





Reagents and conditions: i. Boc_2O , NaOH, H_2O , 96%;
 ii. a. $\text{HP}(\text{OEt})_2$, Et_3N , $(\text{PPh}_3)_4\text{Pd}$, 90°C , b. TMSBr , CH_3CN , 65%;
 iii. Boc_2O , NaOH, $\text{THF}/\text{H}_2\text{O}$, 89%; iv. PhOH , DCC, pyr, 70°C , 71%;
 v. a. NaOH, CH_3CN , 94%; b. Et lactate, DCC, pyr, 70°C , 80%; vi. a. TFA, CH_2Cl_2 ;
 b. **24**, AcOH, NaBH_3CN , EtOH, 33%; vii. 4% HF/ CH_3CN , 88%;
 viii. HCHO, AcOH, NaBH_3CN , EtOH, 67%;
 ix. CH_3CN , DMSO, PBS, porcine liver esterase, 38°C , 21%.

Example 21

Compound 28: To a mixture of 4-bromobenzylamine hydrochloride (15.23 g, 68.4 mmol) in
 5 H_2O (300 mL) was added sodium hydroxide (8.21 g, 205.2 mmol), followed by di-*tert*-butyl
 dicarbonate (16.45g, 75.3 mmol). Reaction mixture was vigorously stirred for 18 h, then
 diluted with EtOAc (500 mL). Organic layer separated and aqueous layer extracted with
 EtOAc (200 mL). Combined organic layer was dried (MgSO_4), concentrated and dried under
 high vacuum to give a white solid (18.7 g, 96%). ^1H NMR (300 MHz, CDCl_3): δ 7.41 (d, J =
 10 8.4 Hz, 2 H), 7.12 (d, J = 8.3 Hz, 2 H), 4.82 (s, 1 H, NH), 4.22 (d, J = 6.1 Hz, 2 H), 1.41 (s, 9
 H).

Example 22

Compound 29: Compound 28 (5.00 g, 17.47 mmol) was coevaporated with toluene. Diethyl
 15 phosphite (11.3 mL, 87.36 mmol) was added and mixture was coevaporated with toluene

(2x). Triethylamine (24.0 mL, 174.7 mmol) was added and mixture was purged with argon for 10 min, then tetrakis(triphenylphosphine) palladium(0) (4.00 g, 3.49 mmol) was added. Reaction mixture was refluxed for 18 h, cooled, concentrated and diluted with EtOAc. Washed with 0.5 N HCl, 0.5 M NaOH, H₂O, brine and dried (MgSO₄). Concentrated and
5 purification (silica gel, 70% EtOAc/Hex) gave an impure reaction product as a yellow oil (6.0 g). This material (6.0 g) was dissolved in anhydrous acetonitrile (30 mL) and cooled to 0°C. Bromotrimethylsilane (11.5 mL, 87.4 mmol) was added and reaction mixture was warmed to room temperature over 15 h. Reaction mixture was concentrated, dissolved in MeOH (50 mL) and stirred for 1.5 h. H₂O (1 mL) was added and mixture stirred for 2 h. Concentrated
10 to dryness and dried under high vacuum, then triturated with Et₂O containing 2% MeOH to give a white solid (3.06 g, 65 %). ¹H NMR (300 MHz, D₂O): δ 7.67 (dd, *J* = 12.9, 7.6 Hz, 2 H), 7.45-7.35 (m, 2 H), 4.10 (s, 2 H); ³¹P NMR (121 MHz, D₂O): δ 12.1.

Example 23

15 Compound 30: Compound **29** (4.78 g, 17.84 mmol) was dissolved in H₂O (95 mL) containing sodium hydroxide (3.57 g, 89.20 mmol). Di-*tert*-butyl dicarbonate (7.63 g, 34.94 mmol) was added, followed by THF (25 mL). The clear reaction mixture was stirred overnight at room temperature then concentrated to ~100 mL. Washed with EtOAc and acidified to pH 1 with 1 N HCl and extracted with EtOAc (7x). Combined organic layer was
20 dried (MgSO₄), concentrated and dried under high vacuum. Trituration with Et₂O gave a white powder (4.56 g, 89%). ¹H NMR (300 MHz, CD₃OD): δ 7.85-7.71 (m, 2 H), 7.39-7.30 (m, 2 H), 4.26 (s, 2 H), 1.46 (s, 9 H); ³¹P NMR (121 MHz, CD₃OD): δ 16.3.

Example 24

25 Compound 31: Compound **30** (2.96 g, 10.32 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL). To this residue was added phenol (9.71 g, 103.2 mmol) and mixture was coevaporated with anhydrous pyridine (2 x 10 mL). Pyridine (50 mL) was added and solution heated to 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (8.51 g, 41.26 mmol) was added and resulting mixture was stirred for 8 h at 70°C. Reaction mixture was cooled
30 and concentrated and coevaporated with toluene. Residue obtained was diluted with EtOAc and the resulting precipitate was removed by filtration. The filtrate was concentrated and purified (silica gel, 20 to 40% EtOAc/Hex, another column 30 to 40% EtOAc/Hex) to give a

white solid (3.20 g, 71%). ¹H NMR (300 MHz, CDCl₃): δ 7.90 (dd, *J* = 13.8, 8.2 Hz, 2 H), 7.41-7.10 (m, 14 H), 5.17 (br s, 1 H, *NH*), 4.35 (d, *J* = 5.2 Hz, 2 H), 1.46 (s, 9 H); ³¹P NMR (121 MHz, CDCl₃): δ 11.8.

5 Example 25

Compound 32: To a solution of compound 31 (3.73 g, 8.49 mmol) in acetonitrile (85 mL) at 0°C was added 1 M NaOH (21.2 mL, 21.21 mmol). Reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature over 4 h. Reaction mixture cooled to 0°C and Dowex (H+) residue was added to pH 2. Mixture was filtered, concentrated and residue
10 obtained was triturated with EtOAc/Hex (1:2) to give a white powder (2.889 g, 94%). This compound (2.00 g, 5.50 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL). The residue was dissolved in anhydrous pyridine (30 mL) and ethyl (S)-lactate (6.24 mL, 55 mmol) and reaction mixture was heated to 70°C. After 5 min, 1,3-dicyclocarbodiimide (4.54 g, 22.0 mmol) was added. Reaction mixture was stirred at 70°C for 5 h, then cooled and
15 concentrated. Residue was dissolved in EtOAc and precipitate was removed by filtration. The filtrate was concentrated and purified (25 to 35% EtOAc/Hex, another column 40% EtOAc/Hex) to give a colorless oil (2.02 g, 80%). ¹H NMR (300 MHz, CDCl₃): δ 7.96-7.85 (m, 2 H), 7.42-7.35 (m, 2 H), 7.35-7.08 (m, 4 H), 5.16-5.00 (m, 1 H), 4.93 (s, 1 H, *NH*), 4.37 (d, *J* = 5.5 Hz, 1 H), 4.21 (q, *J* = 7.3 Hz, 1 H), 4.11 (dq, *J* = 5.7, 2.2 Hz, 1 H), 1.62-1.47 (m, 3
20 H), 1.47 (s, 9 H), 1.27 (t, *J* = 7.3 Hz, 1.5 H), 1.17 (t, *J* = 7.3 Hz, 1.5 H); ³¹P NMR (121 MHz, CDCl₃): δ 16.1, 15.0.

Example 26

Compound 33: Compound 32 (2.02 g, 4.36 mmol) was dissolved in CH₂Cl₂ (41 mL) and
25 cooled to 0°C. To this solution was added trifluoroacetic acid (3.5 mL) and reaction mixture was stirred at 0°C for 1 h, then at room temperature for 3 h. Reaction mixture was concentrated, coevaporated with EtOAc and diluted with H₂O (400 mL). Mixture was neutralized with Amberlite IRA-67 weakly basic resin, then filtered and concentrated. Coevaporation with MeOH and dried under high vacuum to give the TFA amine salt as a
30 semi-solid (1.48 g, 94%). To a solution of the amine (1.48 g, 4.07 mmol) in absolute ethanol (20 mL) at 0°C was added aldehyde 24 (1.39 g, 2.26 mmol), followed by acetic acid (0.14 mL, 2.49 mmol). After stirring for 5 min, sodium cyanoborohydride (0.284 g, 4.52 mmol)

was added and reaction mixture stirred for 30 min at 0°C. Reaction was quenched with saturated NaHCO₃ solution and diluted with EtOAc and H₂O. Aqueous layer was extracted with EtOAc (3x) and combined organic layer was dried (MgSO₄), concentrated and purified (silica gel, 2 to 4% MeOH/CH₂Cl₂) to give white foam (0.727 g, 33%). ¹H NMR (300 MHz, CDCl₃): δ 7.98-7.86 (m, 2 H), 7.71 (d, *J* = 8.6 Hz, 2 H), 7.49 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.98 (d, *J* = 8.8 Hz, 2 H), 5.72 (d, *J* = 5.1 Hz, 1 H), 5.28-5.00 (m, 2 H), 4.30-3.72 (m, 12 H), 3.42-3.58 (m, 1 H), 3.20-2.68 (m, 7 H), 2.25-1.42 (m, 6 H), 1.26 (t, *J* = 7.2 Hz, 1.5 H), 1.17 (t, *J* = 7.2 Hz, 1.5 H), 1.08-0.50 (m, 21 H); ³¹P NMR (121 MHz, CDCl₃): δ 16.1, 15.1.

10 Example 27

Compound 34: To a solution of compound 33 (0.727 g, 0.756 mmol) in acetonitrile (7.6 mL) at 0°C was added 48% hydrofluoric acid (0.152 mL) and reaction mixture was stirred for 40 min at 0°C, then diluted with EtOAc and H₂O. Saturated NaHCO₃ was added and aqueous layer was extracted with EtOAc (2x). Combined organic layer was dried (MgSO₄), concentrated and purified (silica gel, 4 to 5% MeOH/CH₂Cl₂) to give a colorless foam (0.5655 g, 88%). ¹H NMR (300 MHz, CDCl₃): δ 7.95-7.82 (m, 2 H), 7.67 (d, *J* = 8.1 Hz, 2 H), 7.41 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.95 (d, *J* = 7.2 Hz, 2 H), 5.76 (d, *J* = 7.9 Hz, 1 H), 5.67 (d, *J* = 5.0 Hz, 1 H), 5.32-4.98 (m, 2 H), 4.25-3.75 (m, 13 H), 3.25-2.70 (m, 7 H), 2.15-1.76 (m, 3 H), 1.53-1.41 (m, 3 H), 1.25-1.08 (m, 3 H), 0.87 (d, *J* = 4.2 Hz, 6 H); ³¹P NMR (121 MHz, CDCl₃): δ 16.1, 15.0.

Example 28

Compound 35: To a solution of compound 33 (0.560 g, 0.660 mmol) in absolute ethanol (13 mL) at 0°C was added 37% formaldehyde (0.54 mL, 6.60 mmol), followed by acetic acid (0.378 mL, 6.60 mmol). The reaction mixture was stirred at 0°C for 5 min, then sodium cyanoborohydride (0.415 g, 6.60 mmol) was added. Reaction mixture was warmed to room temperature over 2 h, then quenched with saturated NaHCO₃ solution. EtOAc was added and mixture was washed with brine. Aqueous layer was extracted with EtOAc (2x) and combined organic layer was dried (MgSO₄), concentrated and purified (silica gel, 3% MeOH/CH₂Cl₂) to give a white foam (0.384 g, 67%). ¹H NMR (300 MHz, CDCl₃): δ 7.95-7.82 (m, 2 H), 7.71 (d, *J* = 8.4 Hz, 2 H), 7.38 (br s, 2 H), 7.34-7.10 (m, 5 H), 6.98 (d, *J* = 8.8 Hz, 2 H), 5.72 (d, *J* = 5.0 Hz, 1 H), 5.50 (br s, 1 H), 5.19-5.01 (m, 2 H), 4.29-3.75 (m, 10 H),

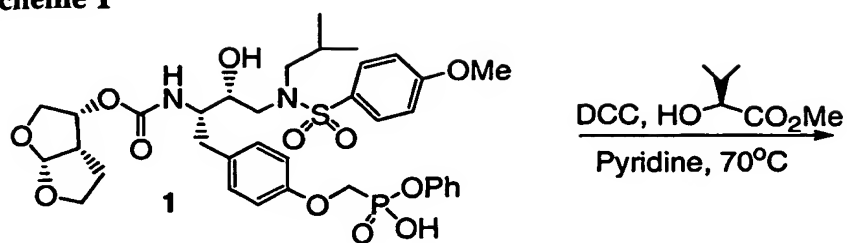
3.85 (s, 3 H), 3.35-2.70 (m, 7 H), 2.23 (s, 3 H), 2.17-1.79 (m, 3 H), 1.54 (d, $J = 6.9$ Hz, 1.5 H), 1.48 (d, $J = 6.8$ Hz, 1.5 H), 1.25 (t, $J = 7.2$ Hz, 1.5 H), 1.16 (t, $J = 7.2$ Hz, 1.5 H), 0.92 (d, $J = 6.6$ Hz, 3 H), 0.87 (d, $J = 6.6$ Hz, 3 H). ^{31}P NMR (121 MHz, CDCl_3): δ 16.0, 14.8.

5 Example 29

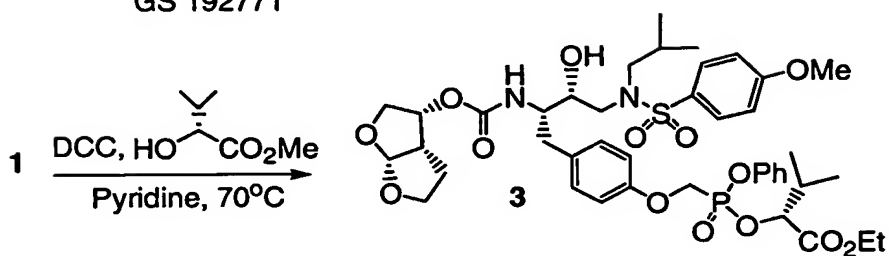
Compound 36: To a solution of compound 35 (44 mg, 0.045 mmol) in acetonitrile (1.0 mL) and DMSO (0.5 mL) was added phosphate buffered saline (pH 7.4, 5.0 mL) to give a cloudy white suspension. Porcine liver esterase (200 μL) was added and reaction mixture was stirred for 48 h at 38°C. Additional esterase (600 μL) was added and reaction was continued for 4 d.

- 10 Reaction mixture was concentrated, diluted with MeOH and the resulting precipitate removed by filtration. Filtrate was concentrated and purified by reverse phase HPLC to give a white powder after lyophilization (7.2 mg, 21%). ^1H NMR (300 MHz, CD_3OD): δ 7.95 (br s, 2 H), 7.76 (d, $J = 8.4$ Hz, 2 H), 7.64 (br s, 2 H), 7.13 (d, $J = 8.7$ Hz, 2 H), 5.68 (d, $J = 5.1$ Hz, 1 H), 5.14 (br s, 1 H), 4.77 (br s, 1 H), 4.35-3.59 (m, 8 H), 3.89 (s, 3 H), 3.45-2.62 (m, 10 H), 2.36-1.86 (m, 3 H), 1.44 (d, $J = 6.3$ Hz, 3 H), 0.92 (d, $J = 6.6$ Hz, 3 H), 0.84 (d, $J = 6.6$ Hz, 3 H);
- 15 ^{31}P NMR (121 MHz, CD_3OD): δ 13.8.

Scheme 1

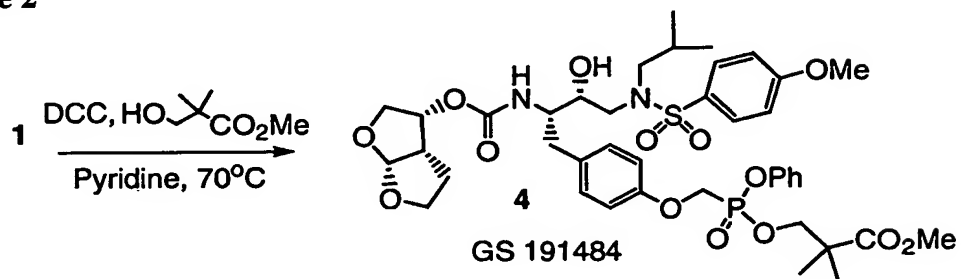


GS 192771

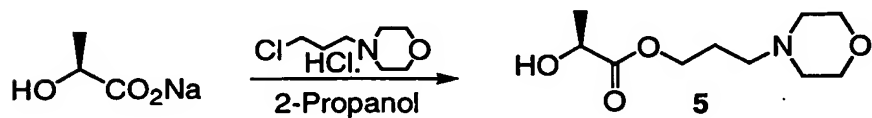


GS 192772

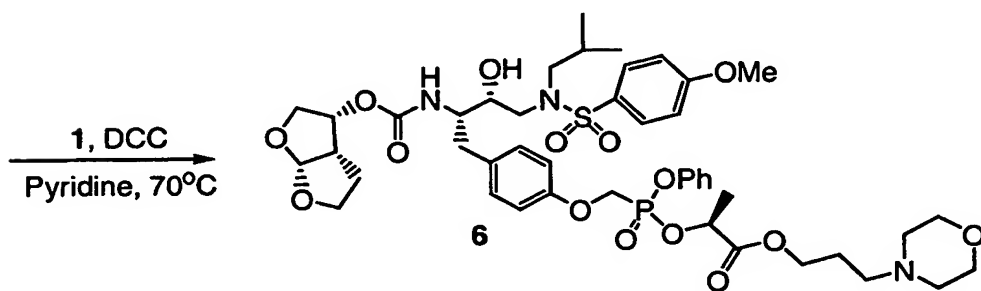
Scheme 2



GS 191484

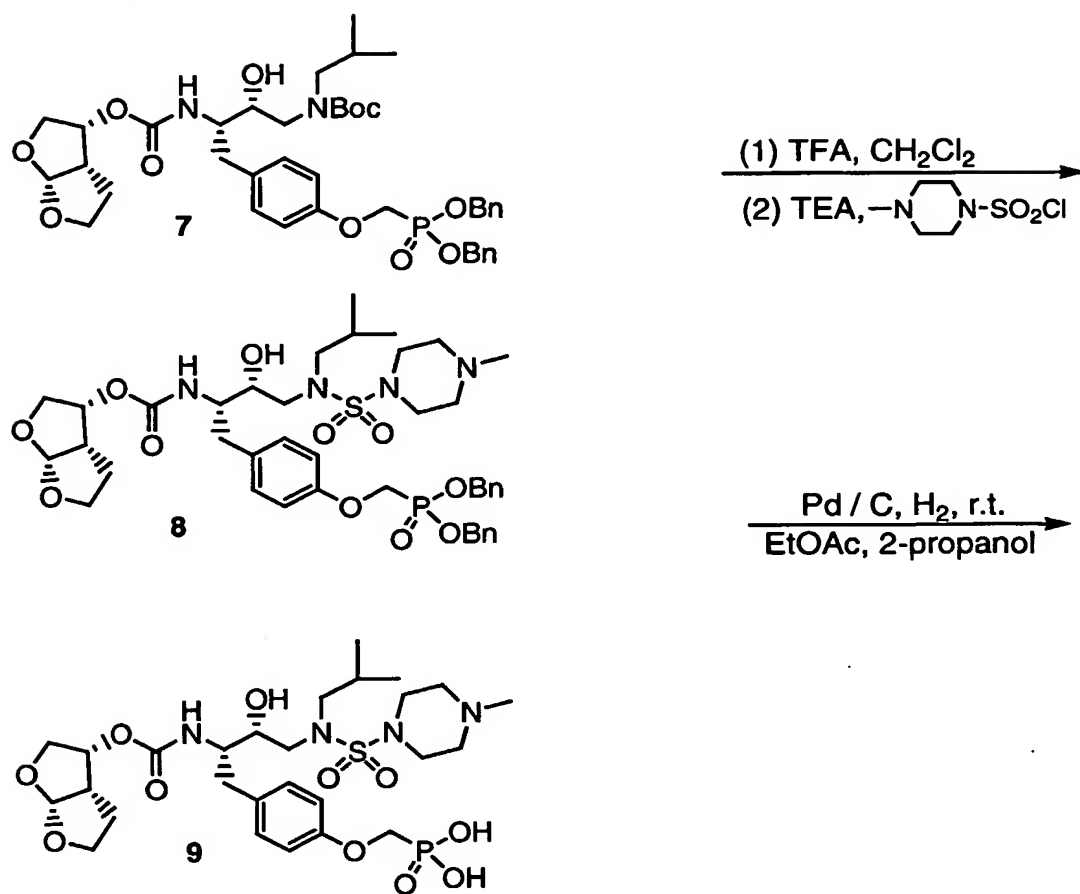


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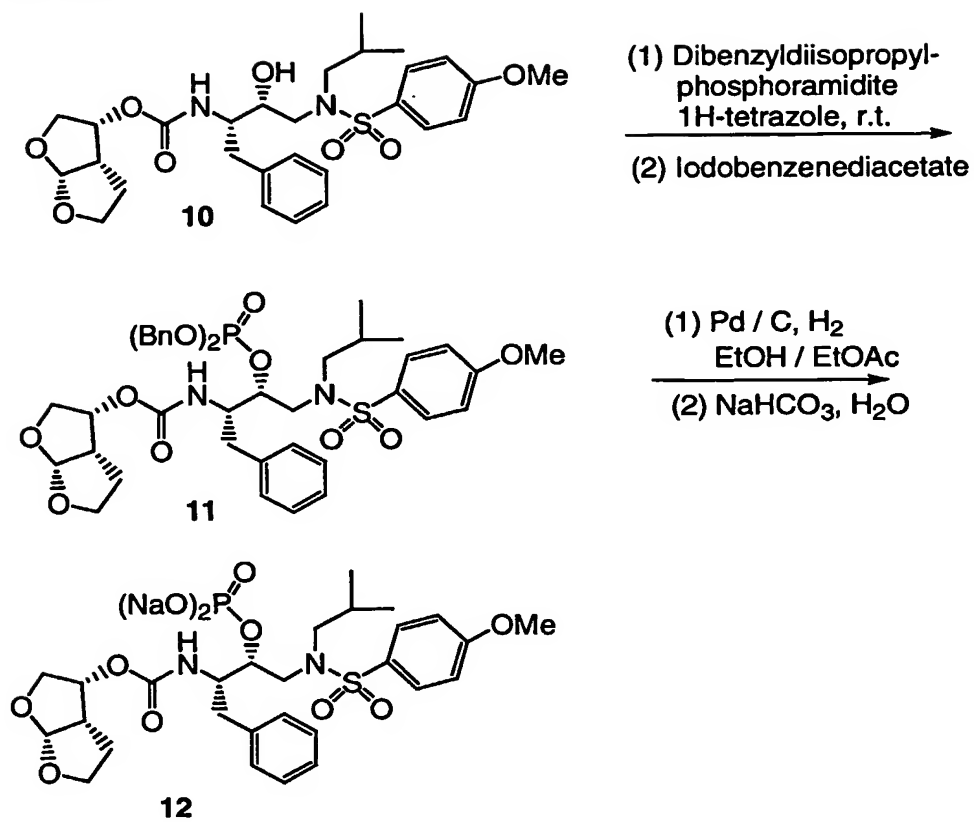


GS 192781

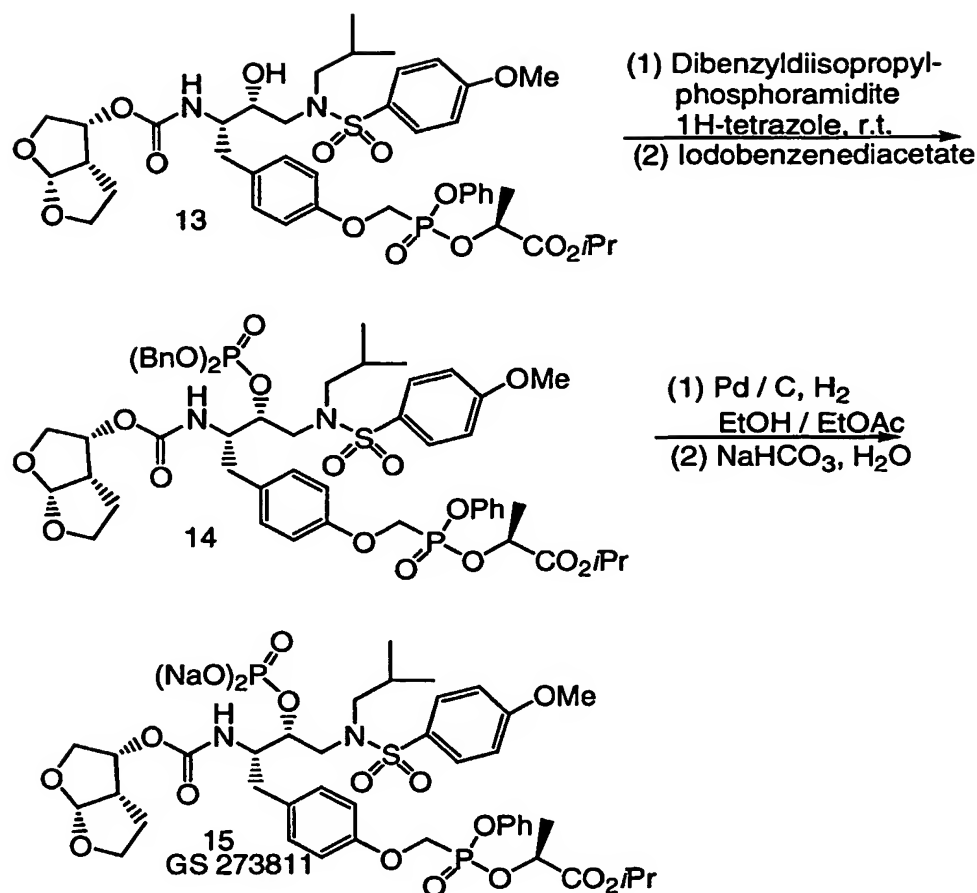
Scheme 3



Scheme 4



Scheme 5

5 Example 1

Monophospholactate 2: A solution of 1 (0.11 g, 0.15 mmol) and α -hydroxyisovaleric acid ethyl-(S)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H₂O, saturated NaCl, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the monophospholactate (35 mg, 28%, GS 192771, 1/1 diastereomeric mixture) as a white solid: ¹H NMR (CDCl₃) δ 7.71 (d, J = 8.7 Hz, 2H), 7.36-7.14 (m, 7H), 6.99 (d, J = 8.7 Hz, 2H), 6.94-6.84 (dd, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.00-4.85 (m, 3H), 4.55 (dd, 1H), 4.41 (dd, 1H), 4.22-4.07 (m, 2H), 3.96-3.68 (m, 9H), 3.12-2.74 (m, 7H), 2.29 (m, 1H), 1.85-1.57

(m, 3H), 1.24 (m, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.9 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.7, 15.1.

Example 2

- 5 Monophospholactate 3: A solution of 1 (0.11 g, 0.15 mmol) and α -hydroxyisovaleric acid ethyl-(R)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off.
- 10 The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H_2O , saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the monophospholactate (35 mg, 28%, GS 192772, 1/1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 7.71 (d, J = 8.7 Hz, 2H), 7.35-7.13 (m, 7H), 6.98 (d, J = 8.7
- 15 Hz, 2H), 6.93-6.83 (dd, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.04-4.85 (m, 3H), 4.54 (dd, 1H), 4.39 (dd, 1H), 4.21-4.06 (m, 2H), 3.97-3.67 (m, 9H), 3.12-2.75 (m, 7H), 2.27 (m, 1H), 1.83-1.57 (m, 3H), 1.26 (m, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.9 (m, 6H); ^{31}P NMR (CDCl_3) δ 17.7, 15.1.

20 Example 3

- Monophospholactate 4: A solution of 1 (0.10 g, 0.13 mmol) and methyl-2,2-dimethyl-3-hydroxypropionate (56 μL , 0.44 mmol) in pyridine (1 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (91 mg, 0.44 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced
- 25 pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H_2O , saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ CH_2Cl_2) to give the monophospholactate (72 mg, 62%, GS 191484) as a white solid: ^1H NMR
- 30 (CDCl_3) δ 7.71 (d, J = 8.7 Hz, 2H), 7.34 (m, 2H), 7.25-7.14 (m, 5H), 7.00 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.05 (m, 2H), 4.38 (d, J = 9.6 Hz, 2H),

4.32-4.20 (m, 2H), 4.00 (m, 2H), 3.87-3.63 (m, 12H), 3.12-2.78 (m, 7H), 1.85-1.67 (m, 3H), 1.20 (m, 6H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{31}P NMR (CDCl_3) δ 16.0.

Example 4

- 5 Lactate 5: To a suspension of lactic acid sodium salt (5 g, 44.6 mmol) in 2-propanol (60 mL) was added 4-(3-chloropropyl)morpholine hydrochloride (8.30 g, 44.6 mmol). The reaction mixture was heated to reflux for 18 h and cooled to room temperature. The solid was filtered and the filtrate was recrystallized from EtOAc / hexane to give the lactate (1.2 g, 12%).

10 Example 5

- Monophospholactate 6: A solution of 1 (0.10 g, 0.13 mmol) and lactate 5 (0.10 g, 0.48 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and H_2O . The EtOAc layer was washed with saturated NaCl, dried with Na_2SO_4 , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ CH_2Cl_2) to give the monophospholactate (30 mg, 24%, GS 192781, 1/1 diastereomeric mixture) as a white solid: ^1H NMR (CDCl_3) δ 7.71 (d, J = 8.7 Hz, 2H), 7.38-7.15 (m, 7H), 7.00 (d, J = 8.7 Hz, 2H), 6.91 (m, 2H), 5.65 (d, J = 3.3 Hz, 1H), 5.18-4.98 (m, 3H), 4.54 (dd, 1H), 4.42 (dd, 1H), 4.2 (m, 2H), 4.00-3.67 (m, 16H), 3.13-2.77 (m, 7H), 2.4 (m, 5H), 1.85-1.5 (m, 5H), 1.25 (m, 2H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{31}P NMR (CDCl_3) δ 17.4, 15.4.

25 Example 6

- Sulfonamide 8: A solution of dibenzylphosphonate 7 (0.1 g, 0.13 mmol) in CH_2Cl_2 (0.5 mL) at 0°C was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (1 mL) and cooled to 0°C. Triethylamine (72 μL , 0.52 mmol) was added followed by the treatment of 4-methylpiperazinylsulfonyl chloride (25 mg, 0.13 mmol). The solution was stirred for 1 h at

0°C and the product was partitioned between CH₂Cl₂ and H₂O. The organic phase was washed with saturated NaCl, dried with Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH₂Cl₂) to give the sulfonamide 8 (32 mg, 30%, GS 273835) as a white solid:

5 ¹H NMR (CDCl₃) δ 7.35 (m, 10H), 7.11 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.2-4.91 (m, 4H), 4.2 (d, J = 10.2 Hz, 2H), 4.0-3.69 (m, 6H), 3.4-3.19 (m, 5H), 3.07-2.75 (m, 5H), 2.45 (m, 4H), 2.3 (s, 3H), 1.89-1.44 (m, 7H), 0.93 (m, 6H); ³¹P NMR (CDCl₃) δ 20.3.

10 Example 7

Phosphonic Acid 9: To a solution of 8 (20 mg, 0.02 mmol) in EtOAc (2 mL) and 2-propanol (0.2 mL) was added 10% Pd/C (5 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (10
15 mg, 64%) as a white solid.

Example 8

Dibenzylphosphonate 11: A solution of 10 (85 mg, 0.15 mmol) and 1*H*-tetrazole (14 mg, 0.20 mmol) in CH₂Cl₂ (2 mL) was treated with Dibenzyl-diisopropylphosphoramidite (60 μL, 0.20 mmol) and stirred at room temperature overnight. The product was partitioned between CH₂Cl₂ and H₂O, dried with Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (85 mg, 0.11 mmol) which was dissolved in CH₃CN (2 mL) and treated with iodobenzenediacetate (51 mg, 0.16 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated.
25 The residue was partitioned between EtOAc and NaHCO₃. The organic layer was washed with H₂O, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the dibenzylphosphonate (45 mg, 52%) as a white solid.

30 Example 9

Disodium Salt of Phosphonic Acid 12: To a solution of 11 (25 mg, 0.03 mmol) in EtOAc (2 mL) was added 10% Pd/C (10 mg). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of

celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid which was dissolved in H₂O (1 mL) and treated with NaHCO₃ (2.53 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (19.77 mg, 95%, GS 273777) as a white solid: ¹H NMR (CD₃OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.27-7.09 (m, 5H), 5.57 (d, J = 5.1 Hz, 1H), 5.07 (m, 1H), 4.87-4.40 (m, 3H), 3.93-3.62 (m, 6H), 3.45-2.6 (m, 6H), 2.0 (m, 2H), 1.55 (m, 1H), 0.95-0.84 (m, 6H).

Example 10

10 Dibenzylphosphonate 14: A solution of 13 (0.80 g, 0.93 mmol) and 1*H*-tetrazole (98 mg, 1.39 mmol) in CH₂Cl₂ (15 mL) was treated with dibenzyl-diisopropylphosphoramidite (0.43 mL, 1.39 mmol) and stirred at room temperature overnight. The product was partitioned between CH₂Cl₂ and H₂O, dried with Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (0.68 g, 67%). To a solution of the dibenzylphosphite (0.39 g, 0.35 mmol) in CH₃CN (5 mL) was added iodobenzenediacetate (0.17 g, 0.53 mmol). The reaction mixture was stirred at room temperature for 2 h and concentrated. The residue was partitioned between EtOAc and NaHCO₃. The organic layer was washed with H₂O, dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH₂Cl₂) to give the dibenzylphosphonate (0.35 g, 88%) as a white solid.

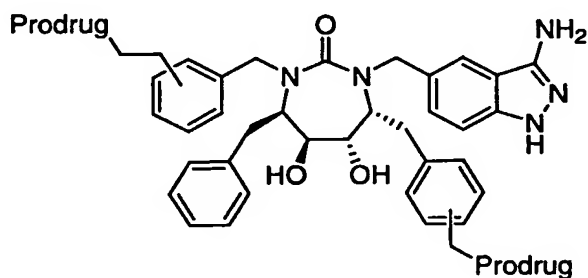
Example 11

Disodium Salt of Phosphonic Acid 15: To a solution of 14 (0.39 g, 0.35 mmol) in EtOAc (30 mL) was added 10% Pd/C (0.10 g). The suspension was stirred under H₂ atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid, which was dissolved in H₂O (3 mL) and treated with NaHCO₃ (58 mg, 0.70 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (0.31 g, 90%, GS 273811) as a white solid: ¹H NMR (CD₃OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.43-7.2 (m, 7H), 7.13 (d, J = 9.0 Hz, 2H), 6.9 (m, 2H), 5.55 (d, J = 4.8 Hz, 1H), 5.07 (m, 2H), 4.87 (m, 1H), 4.64-4.4 (m, 4H), 3.93-3.62 (m, 9H), 3.33-2.63 (m, 5H), 2.11 (m, 1H), 1.6-1.42 (m, 4H), 1.38-1.25 (m, 7H), 0.95 (d, J = 6.3 Hz, 3H), 0.84 (d, J = 6.3 Hz, 3H).

Examples For The Preparation Of Cyclic Carbonyl-Like Phosphonate Protease Inhibitors (CCPPI)

Phosphonamidate Prodrugs

5

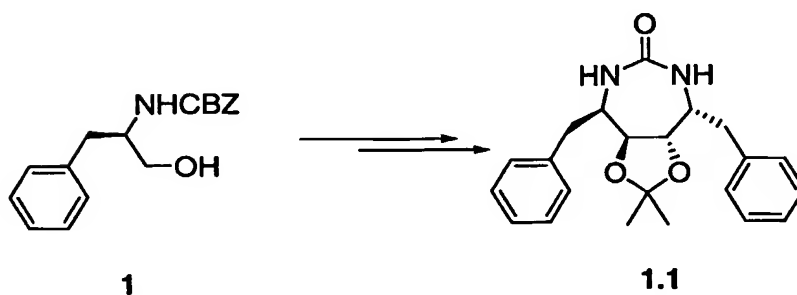


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- | | |
|--------------|-------------------------------|
| Scheme 1-2 | Scaffold Synthesis |
| Scheme 3-10 | P2'-Benzyl ether phosphonates |
| Scheme 11-13 | P2'-Alkyl ether phosphonates |
| Scheme 14-17 | P2'-Benzyl Amide phosphonates |
| Scheme 18-25 | P1-Phosphonates |
| Scheme 50 | Reagents |

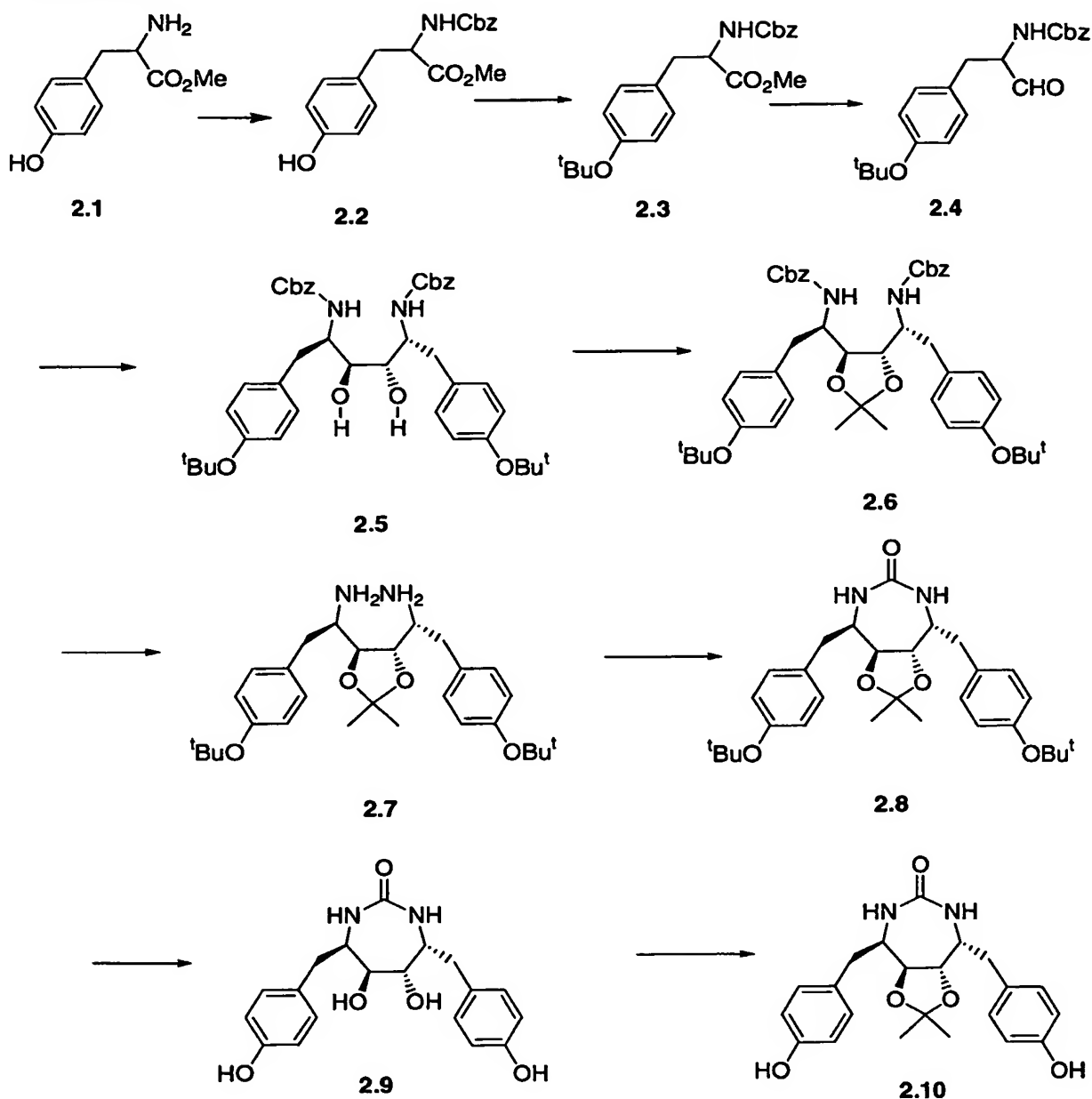
15

Scheme 1



The conversion of 1 to 1.1 is described in J. Org Chem 1996, 61, p444-450

Scheme 2

**2-Benzyloxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionic acid methyl ester (2.3)**

H-D-Tyr-O-me hydrochloride **2.1** (25 g, 107.7 mmol) is dissolved in methylene chloride (150 mL) and aqueous sodium bicarbonate (22 g in 150 mL water), and then cooled to 0°C. To this resulting solution benzyl chloroformate (20 g, 118 mmol) is slowly added. After complete addition, the resulting solution is warmed to room temperature, and is then stirred for 2 h. The organic phase is separated, dried over Na₂SO₄, and concentrated under reduced pressure, to give the crude carbamate **2.2** (35g). The crude CBZ-Tyr-OMe product is

dissolved in methylene chloride (300 mL) containing concentrated H₂SO₄. Isobutene is bubbled through the solution for 6 h. The reaction is then cooled to 0°C, and neutralized with saturated NaHCO₃ aqueous solution. The organic phase is separated, dried, concentrated under reduced pressure, and purified by silica gel column chromatography to afford the tert-butyl ether **2.3** (25.7 g, 62 %).

[2-(4-tert-Butoxy-phenyl)-1-formyl-ethyl]-carbamic acid benzyl ester (2.4)

(Reference J. O. C. 1997, 62, 3884).

To a stirred -78°C methylene chloride solution (60 mL) of **2.3**, DIBAL (82 mL of 1.5 M in toluene, 123 mmol) was added over 15 min. The resultant solution was stirred at -78°C for 30 min. Subsequently, a solution of EtOH/36 % HCl (9/1; 15 mL) is added slowly. The solution is added to a vigorously stirred aqueous HCl solution (600 mL, 1N) at 0°C. The layers are then separated, and the aqueous phase is extracted with cold methylene chloride. The combined organic phases are washed with cold 1N HCl aqueous solution, water, dried over Na₂SO₄, and then concentrated under reduced pressure to give the crude aldehyde **2.4** (20 g, 91 %).

[4-Benzyloxycarbonylamino-1-(4-tert-butoxy-benzyl)-5-(4-tert-butoxy-phenyl)-2,3-dihydroxy-pentyl]-carbamic acid benzyl ester (2.5)

To a slurry of VCl₃(THF)₃ in methylene chloride (150 mL) at room temperature is added Zinc powder (2.9 g, 44 mmol), and the resulting solution is then stirred at room temperature for 1 hour. A solution of aldehyde **2.4** (20 g, 56 mmol) in methylene chloride (100 mL) is then added over 10 min. The resulting solution is then stirred at room temperature overnight, poured into an ice-cold H₂SO₄ aqueous solution (8 mL in 200 mL), and stirred at 0°C for 30 min. The methylene chloride solution is separated, washed with 1N HCl until the washing solution is light blue. The organic solution is then concentrated under reduced pressure (solids are formed during concentration), and diluted with hexane. The precipitate is collected and washed thoroughly with a hexane/methylene chloride mixture to give the diol product **2.5**. The filtrate is concentrated under reduced pressure and subjected to silica gel chromatography to afford a further 1.5 g of **2.5**. (Total = 13 g, 65 %).

[1-{5-[1-Benzyloxycarbonylamino-2-(4-tert-butoxy-phenyl)-ethyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(4-tert-butoxy-phenyl)-ethyl]-carbamic acid benzyl ester (2.6)

Diol 2.5 (5 g, 7 mmol) is dissolved in acetone (120 mL), 2,2-dimethoxypropane (20 mL), and pyridinium p-toluenesulfonate (120 mg, 0.5 mmol). The resulting solution is refluxed for 30 min., and then concentrated under reduced pressure to almost dryness. The resulting mixture is partitioned between methylene chloride and saturated NaHCO₃ aqueous solution, dried, concentrated under reduced pressure, and purified by silica gel column chromatography to afford isopropylidene protected diol 2.6 (4.8 g, 92 %).

4,8-Bis-(4-tert-butoxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxo-5,7-diaza-azulen-6-one(2.8)

The diol 2.6 is dissolved in EtOAc/EtOH (10 mL/2 mL) in the presence of 10 % Pd/C and hydrogenated at atmospheric pressure to afford the diamino compound 2.7. To a solution of crude 2.7 in 1,1,2,2-tetrachloroethane is added 1,1-carboxydiimidazole (1.05 g, 6.5 mmol) at room temperature. The mixture is stirred for 10 min, and the resulting solution is then added dropwise to a refluxing 1,1',2,2'-tetrachloroethane solution (150 mL). After 30 min., the reaction mixture is cooled to room temperature, and washed with 5 % citric acid aqueous solution, dried over Na₂SO₄, concentrated under reduced pressure, and purified by silica gel column chromatography to afford the cyclourea derivative 2.8 (1.92 g, 60 % over 2 steps).

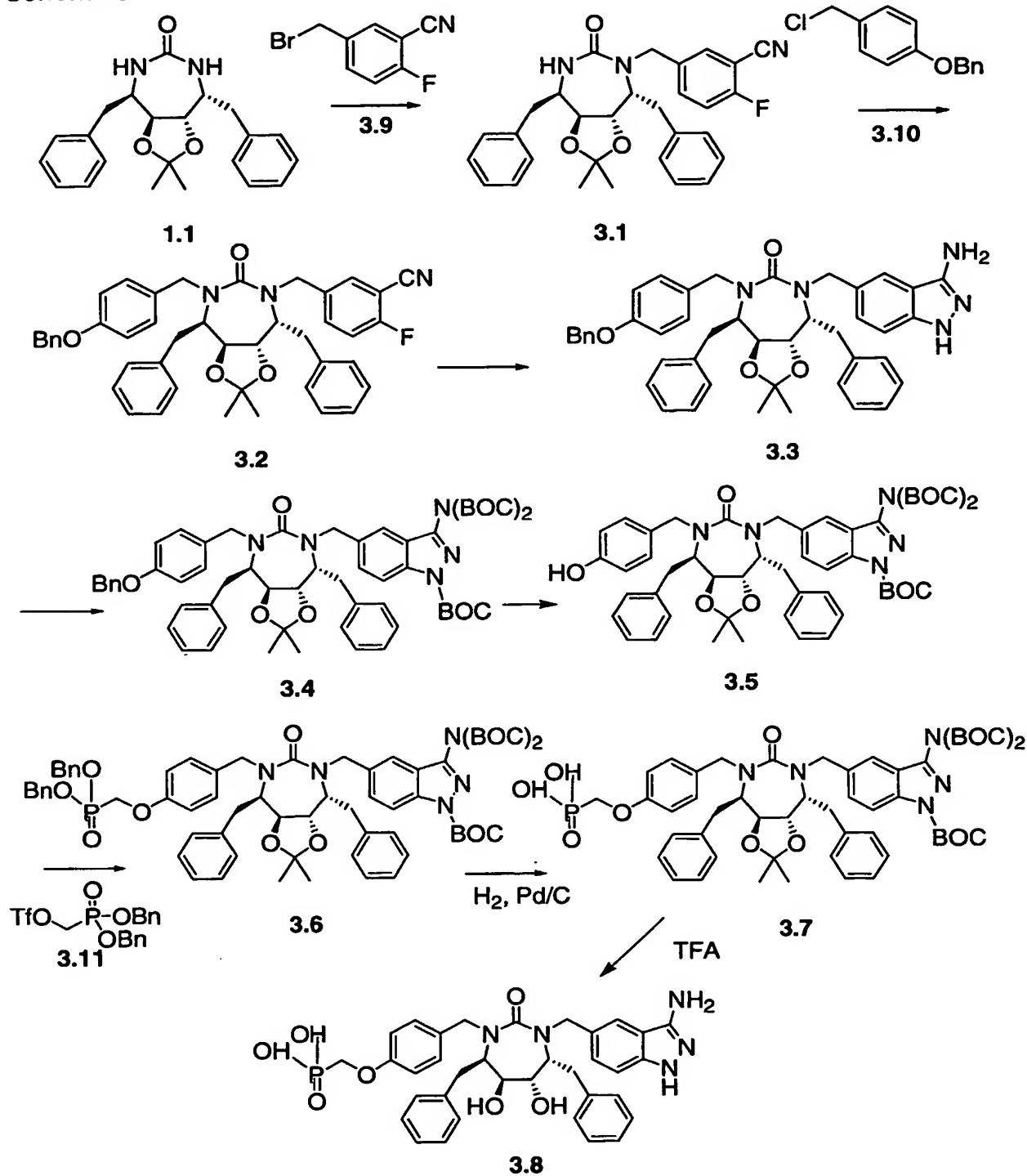
5,6-Dihydroxy-4,7-bis-(4-hydroxy-benzyl)-[1,3]diazepan-2-one (2.9)

Cyclic Urea 2.8 (0.4 g, 0.78 mmol) was dissolved in dichloromethane (3 mL) and treated with TFA (1 mL). The mixture was stirred at room temperature for 2 h upon which time a white solid precipitated. 2 drops of water and methanol (2 mL) were added and the homogeneous solution was stirred for 1 h and concentrated under reduced pressure. The crude solid, 2.9, was dried overnight and then used without further purification.

4,8-Bis-(4-hydroxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxo-5,7-diaza-azulen-6-one (2.10)

Diol 2.9 (1.8 g, 5.03 mmol) was dissolved in DMF (6 mL) and 2,2-dimethoxypropane (12 mL). P-TsOH (95 mg) was added and the mixture stirred at 65°C for 3 h. A vacuum was applied to remove water and then the mixture was stirred at 65°C for a further 1 h. The excess dimethoxypropane was then distilled and the remaining DMF solution was then

allowed to cool. The solution of acetonide **2.10** can then be used without further purification in future reactions.

Scheme 3

3-Cyano-4-fluorobenzyl urea 3.1 : A solution of urea **1.1** (1.6 g, 4.3 mmol) in THF was treated with sodium hydride (0.5 g of 60 % oil dispersion, 13 mmol). The mixture was stirred at room temperature for 30 min and then treated with 3-cyano-4-fluorobenzyl bromide **3.9** (1.0 g, 4.8 mmol). The resultant solution was stirred at room temperature for 3 h,
5 concentrated under reduced pressure, and then partitioned between CH₂Cl₂ and saturated brine solution containing 1 % citric acid. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 15-25% ethyl acetate in hexanes to yield urea **3.1** (1.5 g, 69 %) as a white form.

10

Benzyl ether 3.2 : A solution of **3.1** (0.56 g, 1.1 mmol) in DMF (5 mL) was treated with sodium hydride (90 mg of 60 % oil dispersion, 2.2 mmol) and the resultant mixture stirred at room temperature for 30 min. 4-Benzyloxy benzyl chloride **3.10** (0.31 g, 1.3 mmol) was added and the resultant solution stirred at room temperature for 3 h. The mixture was
15 concentrated under reduced pressure and then partitioned between CH₂Cl₂ and saturated brine solution. The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel eluting with 1-10% ethyl acetate in hexanes to yield compound **3.2** (0.52 g, 67 %) as white form.

20 **Indazole 3.3**: Benzyl ether **3.2** (0.51 g, 0.73 mmol) was dissolved in n-butanol (10 mL) and treated with hydrazine hydrate (1 g, 20 mmol). The mixture was refluxed for 4 h and then allowed to cool to room temperature. The mixture was concentrated under reduced pressure and the residue was then partitioned between CH₂Cl₂ and 10 % citric acid solution. The organic phase was separated, concentrated under reduced pressure, and then purified by silica
25 gel column eluting with 5% methanol in CH₂Cl₂ to afford indazole **3.3** (0.42 g, 82 %) as white solid.

Boc-indazole 3.4 : A solution of indazole **3.3** (0.4 g, 0.59 mmol) in CH₂Cl₂ (10 mL) was treated with diisopropylethylamine (0.19 g, 1.5 mmol), DMAP (0.18 g, 1.4 mmol), and di-
30 tert-butyl dicarbonate (0.4 g, 2 mmol). The mixture was stirred at room temperature for 3 h and then partitioned between CH₂Cl₂ and 5 % citric acid solution. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The

residue was purified by silica gel eluting with 2% methanol in CH_2Cl_2 to afford **3.4** (0.42 g, 71 %).

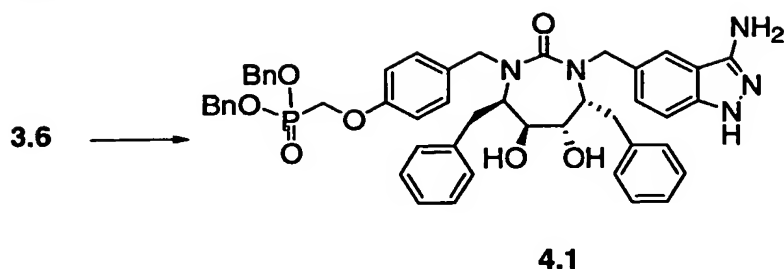
Phenol 3.5 : A solution of **3.4** (300 mg, 0.3 mmol) in ethyl acetate (10 mL) and methanol (10 mL) was treated with 10 % Pd/C (40 mg) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **3.5** as a white powder. This was used without further purification.

Dibenzyl ester 3.6 : A solution of **3.5** (0.1 mmol) in THF (5 mL) was treated with dibenzyl triflate **3.11** (90 mg, 0.2 mmol), and cesium carbonate (0.19 g, 0.3 mmol). The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 20-40% ethyl acetate in hexanes to afford **3.6** (70 mg, 59 %). ^1H NMR (CDCl_3): δ 8.07 (d, 1H), 7.20-7.43 (m, 16H), 7.02-7.15 (m, 8 H), 6.80 (d, 2H), 5.07-5.18 (m, 4H), 5.03 (d, 1H), 4.90 (d, 1H), 4.20 (d, 2H), 3.74-3.78 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H) 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.26 (s, 6H); ^{31}P NMR (CDCl_3): 20.5 ppm.

Phosphonic acid 3.7: A solution of dibenzylphosphonate **3.6** (30 mg) in EtOAc (10 mL) was treated with 10% Pd/C (10 mg) and the mixture was stirred under a hydrogen atmosphere (balloon) for 3 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford phosphonic acid **3.7**. This was used without further purification.

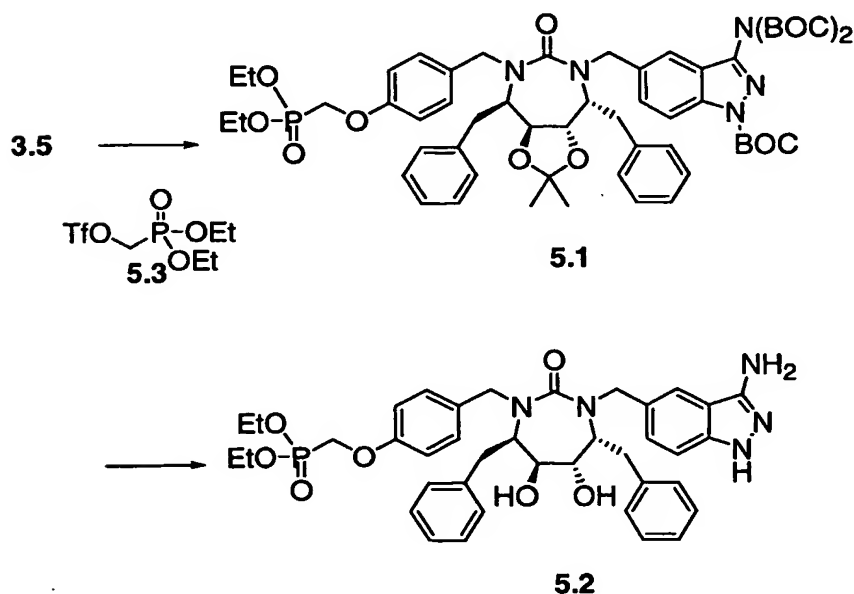
Phosphonic acid 3.8: The crude phosphonic acid **3.7** was dissolved in CH_2Cl_2 (2 mL) and treated with trifluoroacetic acid (0.4 mL). The resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and then purified by preparative HPLC (35 % CH_3CN /65 % H_2O) to afford the phosphonic acid **3.8** (9.4 mg, 55 %). ^1H NMR (CD_3OD): δ 7.71 (s, 1H), 7.60 (d, 1H), 6.95-7.40 (m, 15H), 4.65 (d, 2H), 4.17 (d, 2H), 3.50-3.70 (m, 3H), 3.42 (d, 1H), 2.03-3.14 (m, 6H); ^{31}P NMR (CDCl_3): 17.30

Scheme 4



Dibenzylphosphonate 4.1: A solution of 3.6 (30 mg, 25 μ mol) in CH_2Cl_2 (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to afford 4.1 (5 mg, 24%). ^1H NMR (CDCl_3): δ 6.96-7.32 (m, 25H), 6.95 (d, 2H), 5.07-5.18 (m, 4H), 4.86 (d, 1H), 4.75 (d, 1H), 4.18 (d, 2H), 3.40-3.62 (m, 4H), 3.25 (d, 1H), 2.80-3.15 (m, 6H); ^{31}P NMR (CDCl_3) 20.5 ppm; MS : 852 (M + H), 874 (M + Na).

Scheme 5

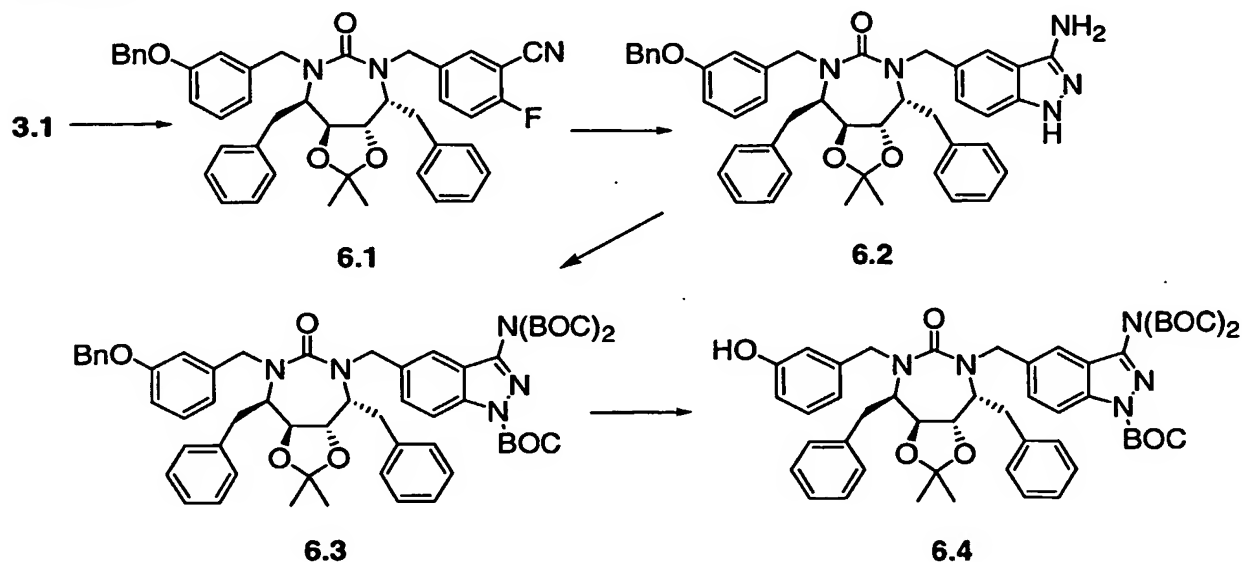


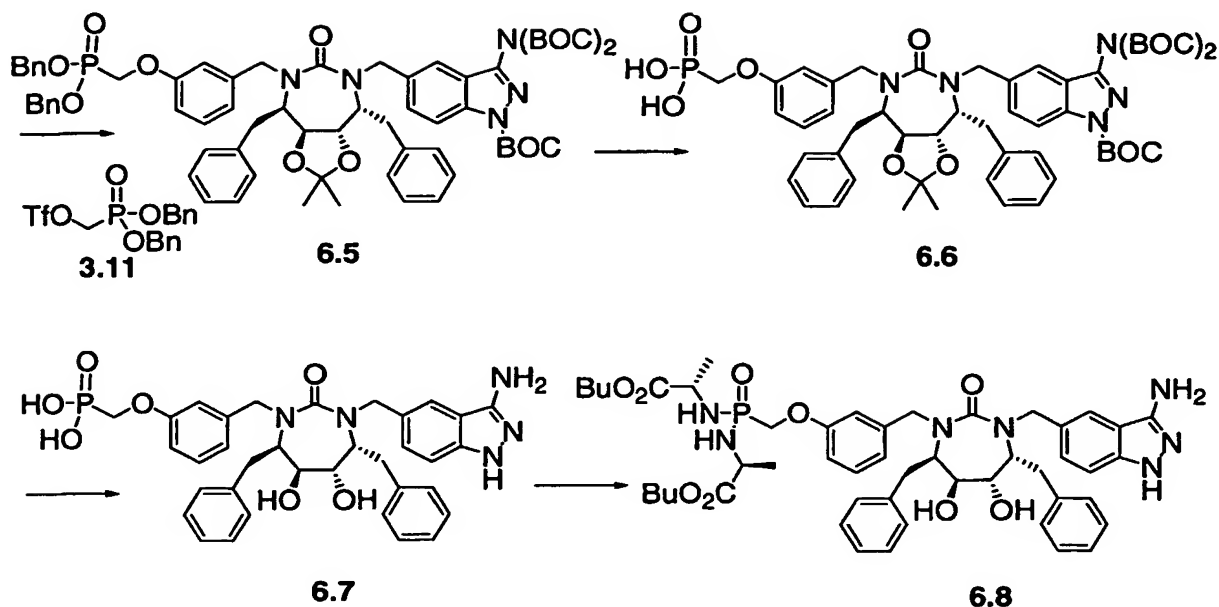
Diethylphosphonate 5.1: A solution of phenol 3.5 (48 mg, 52 μ mol) in THF (5 mL) was treated with triflate 5.3 (50 mg, 165 μ mol), and cesium carbonate (22 mg, 0.2 mmol). The

resultant mixture was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 7% methanol in CH_2Cl_2 to afford **5.1** (28 mg, 50 %). ^1H NMR (CDCl_3): δ 8.06 (d, 1H), 7.30-7.43 (m, 7H), 7.02-7.30 (m, 7 H), 6.88 (d, 2H), 5.03 (d, 1H), 4.90 (d, 1H), 4.10-4.25 (m, 6H), 3.64-3.80 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H) 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.20-1.50 (m, 30H); ^{31}P NMR (CDCl_3): 18.5 ppm; MS :1068 (M + H), 1090 (M + Na).

- 10 **Diethylphosphonate 5.2:** A solution of **5.1** (28 mg, 26 μmol) in CH_2Cl_2 (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 hrs. The mixture was concentrated under reduced pressure and the residue was purified by silica gel to afford **5.2** (11 mg, 55 %). ^1H NMR ($\text{CDCl}_3 + 10\%$ CD_3OD): δ 6.96-7.35 (m, 15H), 6.82 (d, 2H), 4.86(d, 1H), 4.75 (d, 1H), 4.10-4.23 (M, 6H), 3.40-3.62 (m, 4H), 2.80-3.20 (m), 1.31 (t, 6 H); ^{31}P NMR ($\text{CDCl}_3 + 10\%$ CD_3OD): 19.80 ppm; MS : 728 (M + H).

Scheme 6





- 3-Benzyloxybenzyl urea 6.1 :** The urea 3.1 (0.87 g, 1.7 mmol) was dissolved in DMF and treated with sodium hydride (60% dispersion, 239 mg, 6.0 mmol) followed by m-
 5 benzyloxybenzylbromide 6.9 (0.60 g, 2.15 mmol). The mixture was stirred for 5 h and then diluted with ethyl acetate. The solution was washed with water, brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 25% ethyl acetate in hexanes to afford urea 6.1 (0.9 g, 75%).
- Indazole 6.2:** The urea 6.1 (41 mg, 59 μ mol) was dissolved in n-butanol (1.5 mL) and treated with hydrazine hydrate (100 μ L, 100 mmol). The mixture was refluxed for 2 h and then allowed to cool. The mixture was diluted with ethyl acetate, washed with 10% citric acid solution, brine, saturated NaHCO₃, and finally brine again. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure to give the crude
 10 product 6.2 (35 mg, 83%). (Chem. Biol. 1998, 5, 597-608).

- Boc-indazole 6.3 :** The indazole 6.2 (1.04 g, 1.47 mmol) was dissolved in CH₂Cl₂ (20 mL) and treated with di-t-butyl dicarbonate (1.28 g, 5.9 mmol), DMAP (0.18 g, 1.9 mmol) and DIPEA (1.02 mL, 9.9 mmol). The mixture was stirred for 3 h and then diluted with ethyl
 20 acetate. The solution was washed with 5% citric acid solution, NaHCO₃, brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to give 6.3 (0.71 g, 49%).

Phenol 6.4 : Compound **6.3** (20 mg, 0.021 mmol) was dissolved in MeOH (1 mL) and EtOAc (1 mL) and treated with 10% Pd/ C catalyst (5 mg). The mixture was stirred under a hydrogen atmosphere (balloon) until completion. The catalyst was removed by filtration and
5 the filtrate concentrated under reduced pressure to afford compound **6.4** (19 mg, 100%).

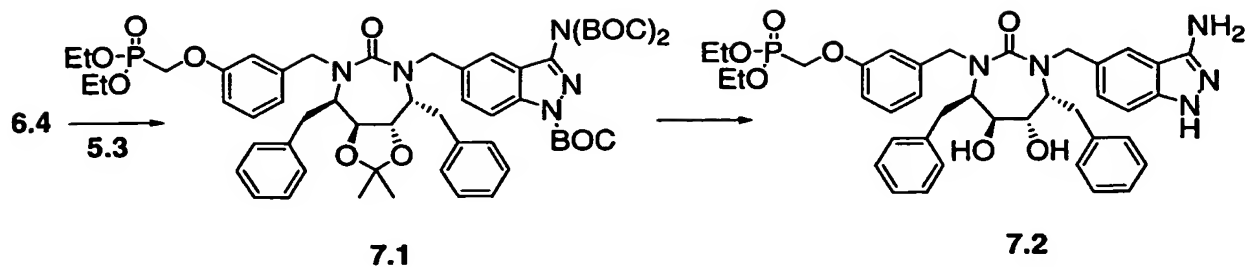
Dibenzyl phosphonate 6.5: A solution of compound **6.4** (0.34 g, 0.37 mmol) in acetonitrile (5 mL) was treated with Cs₂CO₃ (0.36 g, 1.1 mmol) and triflate **3.11** (0.18 mL, 0.52 mmol). The reaction mixture was stirred for 1 h. The reaction mixture was filtered and the filtrate
10 was then concentrated under reduced pressure. The residue was re-dissolved in EtOAc, washed with water, saturated NaHCO₃, and finally brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with hexane: EtOAc (1:1) to afford compound **6.5** (0.32 g, 73%).

Phosphonic acid 6.6: Compound **6.5** (208 mg, 0.174 mmol) was treated in the same manner as benzyl phosphonate **3.6** in the preparation of phosphonate diacid **3.7**, except MeOH was used as the solvent, to afford compound **6.6** (166 mg, 94%).

Phosphonic acid 6.7: Compound **6.6** (89 mg, 0.088 mmol) was treated according to the
20 conditions described in Scheme 3 for the conversion of **3.7** into **3.8**. The residue was purified by preparative HPLC eluting with a gradient of 90% methanol in 100 mM TEA bicarbonate buffer and 100% TEA bicarbonate buffer to afford phosphonic acid **6.7** (16 mg, 27%)

Bisamidate 6.8 : Triphenylphosphine (112 mg, 0.43 mmol) and aldrithiol-2 (95 mg, 0.43
25 mmol) were mixed in dry pyridine (0.5 mL). In an adjacent flask the diacid **6.7** (48 mg, 0.71 mmol) was suspended in dry pyridine (0.5 mL) and treated with DIPEA (0.075 mL 0.43 mmol) and L-AlaButyl ester hydrochloride (78 mg, 0.43 mmol) and finally the triphenylphosphine, aldrithiol-2 mixture. The reaction mixture was stirred under nitrogen for 24 h then concentrated under reduced pressure. The residue was purified by preparative
30 HPLC eluting with a gradient of 5% to 95% acetonitrile in water. The product obtained was then further purified by silica gel eluting with CH₂Cl₂ : MeOH (9:1) to give compound **6.8** (9 mg, 14%).

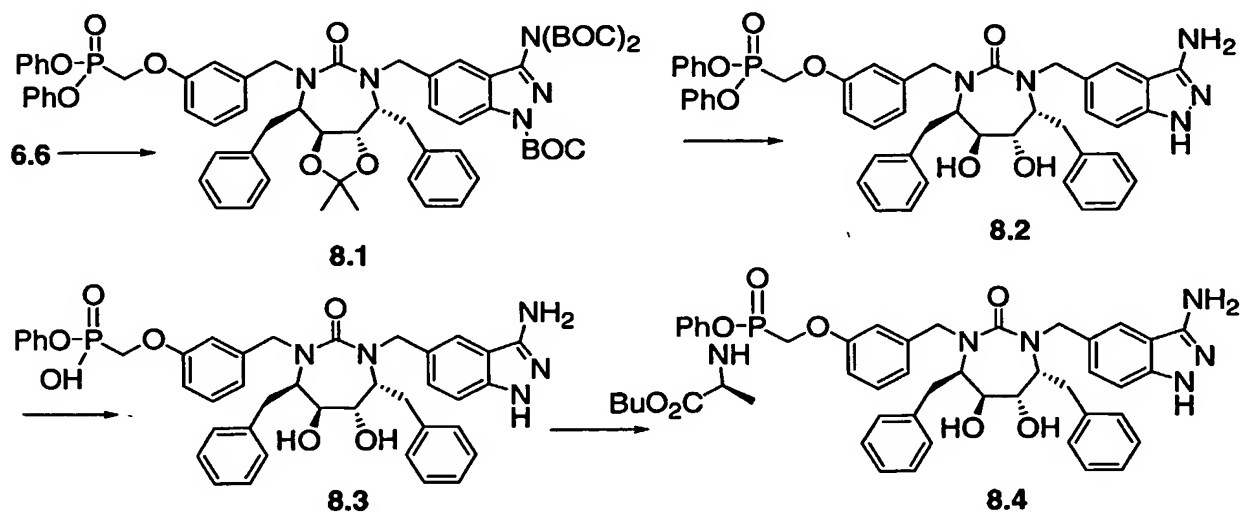
Scheme 7



- 5 **Diethyl phosphonate 7.1** : Compound 6.4 (164 mg, 0.179 mmol) was treated according to the procedure used to generate compound 6.5 except triflate 5.3 was used in place of triflate 3.11 to afford compound 7.1 (142 mg, 74%).

- 10 **Diethylphosphonate 7.2** : Compound 7.1 (57 mg, 0.053 mmol) was treated according to the conditions used to form 6.7 from 6.6. The residue formed was purified by silica gel eluting with CH_2Cl_2 : MeOH (9:1) to afford compound 7.2 (13 mg, 33%).

Scheme 8



15

- Diphenylphosphonate 8.1**: A solution of 6.6 (0.67g, 0.66 mmol) in pyridine (10 mL) was treated with phenol (0.62 g, 6.6 mmol) and DCC (0.82 mg, 3.9 mmol). The resultant mixture was stirred at room temperature for 5 min and then the solution was heated at 70°C for 3 h.
- 20 The mixture was allowed to cool to room temperature and then diluted with EtOAc and water

(2 mL). The resultant mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure. The residue was triturated with CH_2Cl_2 , and the white solid that formed was removed by filtration. The filtrate was concentrated under reduced pressure and the resultant residue was purified by silica gel eluting with 30% ethyl acetate in hexanes to yield **8.1** (0.5 g, 65 %). ^1H NMR (CDCl_3): δ 8.08 (d, 1H), 7.41 (d, 1H), 7.05-7.35 (m, 22H), 6.85 (d, 2H), 6.70 (s, 1H), 5.19 (d, 1H), 5.10 (d, 1H), 4.70 (d, 2H), 3.70-3.90 (m, 4H), 3.20 (d, 1H), 3.11 (d, 1H), 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.30 (s, 6H); ^{31}P NMR (CDCl_3): 12.43 ppm

Diphenylphosphonate 8.2 : A solution of **8.1** (0.5 g, 0.42 mmol) in CH_2Cl_2 (4 mL) was treated with TFA (1 mL) and the resultant mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and azeotroped twice with CH_3CN . The residue was purified by silica gel eluting with 5% methanol in CH_2Cl_2 to afford diphenylphosphonate **8.2** (0.25 g, 71 %). ^1H NMR (CDCl_3): δ 7.03-7.40 (m, 21H), 6.81-6.90 (m, 3H), 4.96 (d, 1H), 4.90 (d, 1H), 4.60-4.70 (m, 2H), 3.43-3.57 (m, 4H), 3.20 (d, 1H), 2.80-2.97 (m, 5H); ^{31}P NMR (CDCl_3): 12.13 ppm; MS : 824 (M + H).

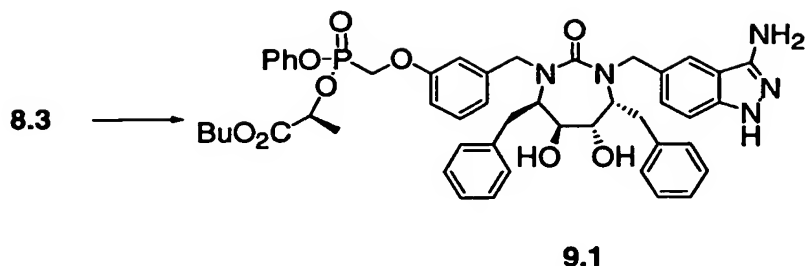
Monophenol 8.3 : The monophenol **8.3** (124 mg, 68 %) was prepared from the diphenol **8.2** by treating with 1N NaOH in acetonitrile at 0°C .

20

Monoamidate 8.4 : To a pyridine solution (0.5 mL) of **8.3** (40 mg, 53 μmol), n-butyl amidate HCl salt (116 mg, 640 μmol), and DIPEA (83 mg, 640 μmol) was added a pyridine solution (0.5 mL) of triphenyl phosphine (140 mg, 640 μmol), and aldrithiol-2 (120 mg, 640 μmol). The resulting solution was stirred at 65°C overnight, worked up, and purified by preparative TLC twice to give **8.4** (1.8 mg). δ 4.96 (d, 1H), 4.90 (d, 1H), 4.30-4.6 (m, 2H), 3.9-4.2 (m, 2H), 3.6-3.70 (m, 4H), 3.2-3.3 (d, 1H), 2.80-3.1 (m, 4H); MS: 875 (M + H) & 897 (M + Na)

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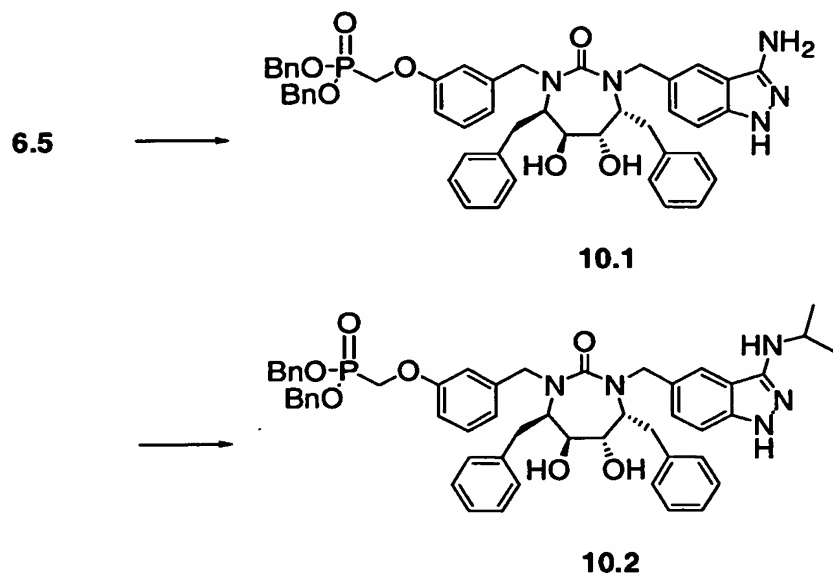
Scheme 9



- 5 **Monolactate 9.1:** The monolactate **9.1** is prepared from **8.3** using the conditions described above for the preparation of the monoamidate **8.4** except n-butyl lactate was used in place of n-butyl amidate HCl salt.

Scheme 10

10

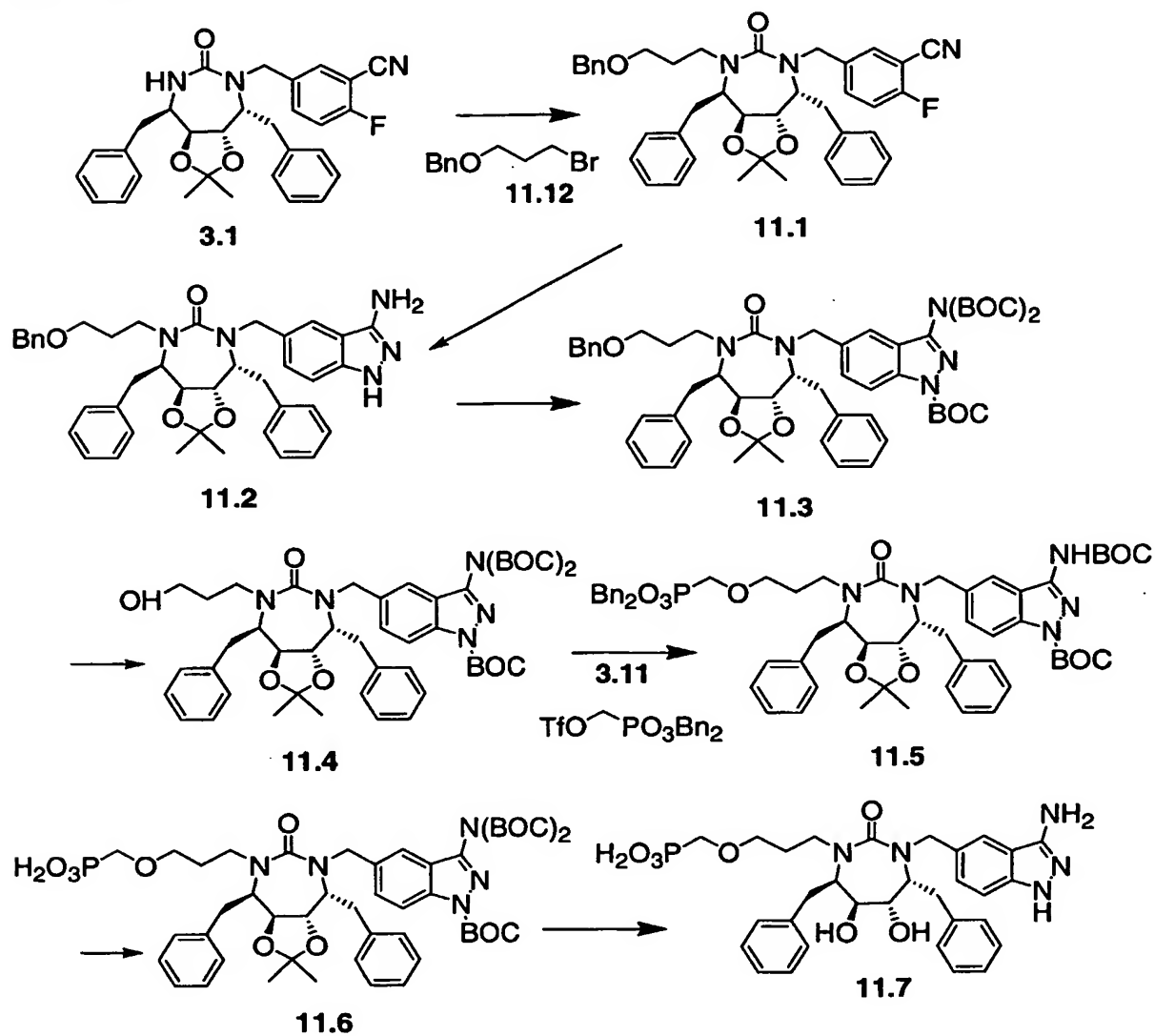


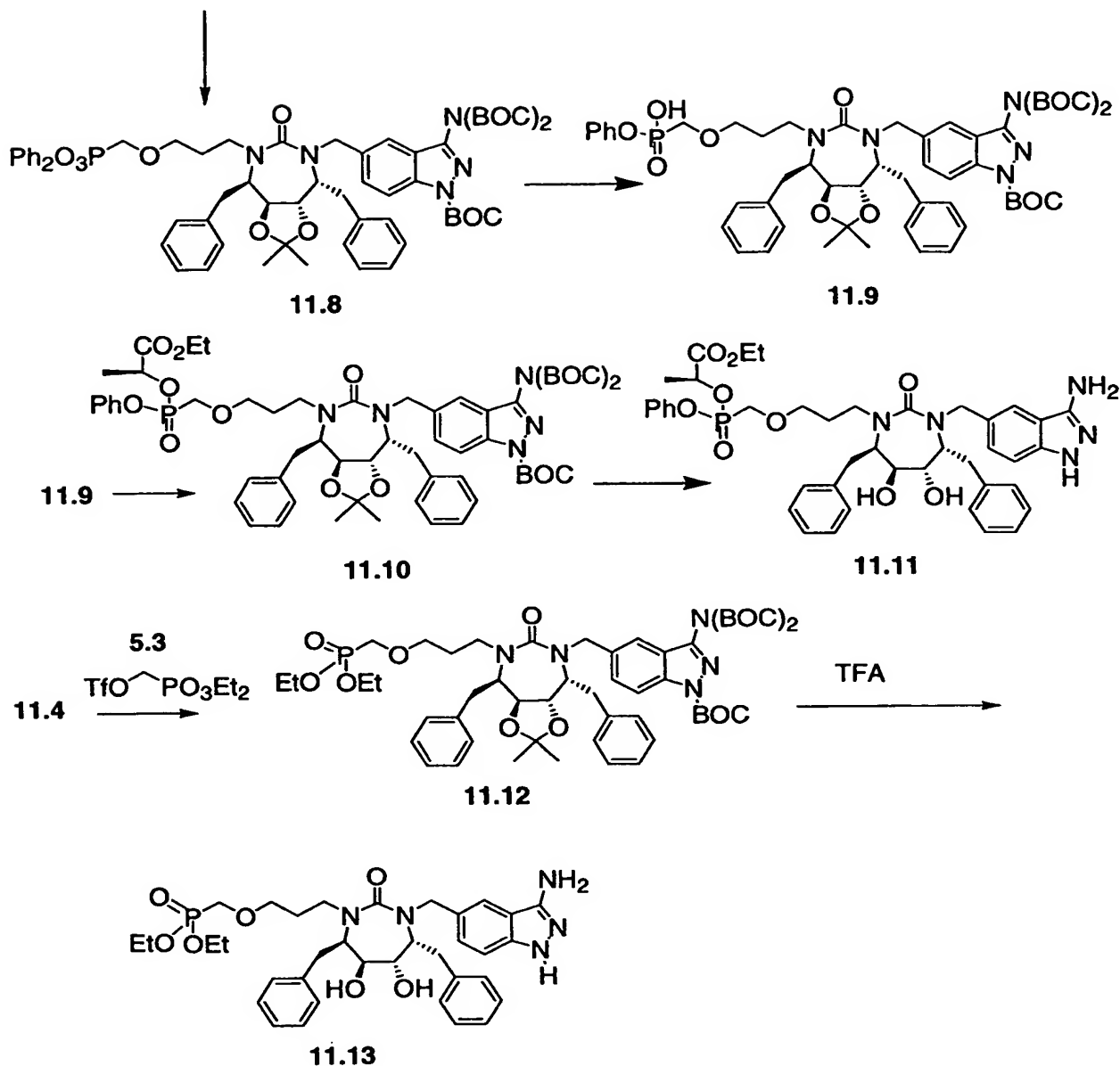
- 15 **Dibenzylphosphonate 10.1:** Compound **6.5** (16 mg, 0.014 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C . TFA (1 mL) was added and the reaction mixture was stirred for 0.5 h. The mixture was then allowed to warm to room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and azeotroped with toluene. The residue was purified by silica gel eluting with CH_2Cl_2 : MeOH (9:1) to afford compound **10.1** (4 mg, 32%).

Isopropylamino indazole 10.2 : Compound **10.1** (30 mg, 0.35 mmol) was treated with acetone according to the method of Henke et al. (J. Med Chem. 40 17 (1997) 2706-2725) to yield **10.2** as a crude residue. The residue was purified by silica gel eluting with CH₂Cl₂ : MeOH (93:7) to afford compound **10.2** (3.4 mg, 10%).

5

Scheme 11





Benzyl ether 11.1: A DMF solution (5 mL) of 3.1 (0.98 g, 1.96 mmol) was treated with NaH (0.24 g of 60 % oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol), and benzoxypropyl bromide (0.55 g, 2.4 mmol). After the reaction for 3 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give 11.1 (0.62 g, 49 %).

Aminoindazole 11.2: A n-butanol solution (10 mL) of 11.1 (0.6 g, 0.92 mmol) and hydrazine hydrate (0.93 g, 15.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude 11.2 (~0.6 g).

Tri-BOC-Aminoindazole 11.3: A methylene chloride solution (10 mL) of crude **11.2**, DIPEA (0.36 g, 2.8 mmol), (BOC)₂O (0.73 g, 3.3 mmol), and DMAP (0.34 g, 2.8 mmol) was stirred for 5 h at room temperature, partitioned between methylene chloride and 5 % citric acid solution, dried, purified by silica gel column chromatography to give **11.3** (0.51 g, 58 %, 2 steps).

3-Hydroxypropyl cyclic urea 11.4: An ethyl acetate/ethanol solution (30 mL/5 mL) of **11.3** (0.5 g, 0.52 mmol) was hydrogenated at 1 atm in the presence of 10 % Pd/C (0.2 g) for 4 h. The catalyst was removed by filtration. The filtrate was then concentrated under reduced pressure to afford crude **11.4** (0.44 g, 98 %).

Dibenzyl phosphonate 11.5: A THF solution (3 mL) of **11.4** (0.5 g, 0.57 mmol) and triflate dibenzyl phosphonate **3.11** (0.37 g, 0.86 mmol) was cooled to -3°C, followed by addition of n-BuLi (0.7 mL of 2.5 M hexane solution, 1.7 mmol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure. The residue was redissolved in methylene chloride (10 mL), and reacted with (BOC)₂O (0.15 g, 0.7 mmol) in the presence of DMAP (0.18 g, 0.57 mmol), DIPEA (0.18 g, 1.38 mmol) for 2 h at room temperature. The reaction mixture was worked up, and purified by silica gel chromatography to give **11.5** (0.25 g, 43 %).

Phosphonic diacid 11.7: An ethyl acetate solution (2 mL) of **11.5A** (11 mg, 10.5 µmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 6 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give crude **11.6**. The crude **11.6** was redissolved in methylene chloride (1 mL) and treated with TFA (0.2 mL) for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by HPLC to give **11.7** (2 mg, 30%).

NMR (CD₃OD): δ 7.1-7.3 (m, 11H), 7.0-7.1 (d, 2H), 4.95 (d, 1H), 3.95-4.1 (d, 1H), 2.9-3.3 (m, 4H), 2.3-2.45 (m, 1H), 1.6-1.8 (m, 2H). P NMR (CD₃OD): 15.5 ppm. MS: 624 (M + 1).

Diphenyl phosphonate 11.8: A pyridine solution (1 mL) of **11.6** (0.23 g, 0.23 mmol), phenol (0.27 g, 2.8 mmol), and DCC (0.3 g, 1.4 mmol) was stirred for 5 min. at room temperature, then reacted at 70°C for 3 h. The reaction mixture was cooled to room

temperature, concentrated under reduced pressure, and purified by silica gel column chromatograph to afford **11.8** (0.11g, 41 %).

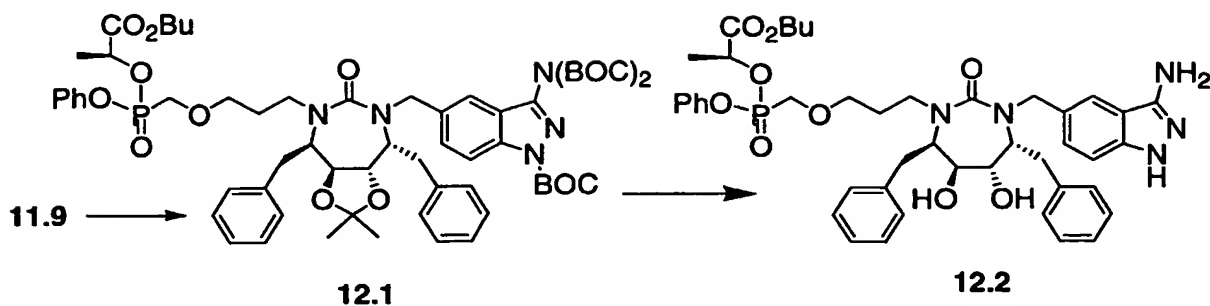
Monophenyl phosphonate 11.9: An acetonitrile solution (2 mL) of **11.8** (0.12 g, 0.107 mmol) at 0°C was treated with 1N sodium hydroxide aqueous solution (0.2 mL) for 1.5 h., then acidified with Dowex (50wx8-200, 120 mg). The Dowex was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was triturated with 10 % EtOAc/90 % hexane twice to afford **11.9** (90 mg, 76 %) as a white solid .

Mono-ethyl lactate phosphonate 11.10: A pyridine solution (0.3 mL) of **11.9** (33 mg, 30 µmol), ethyl lactate (41 mg, 340 µmol), and DCC (31 mg, 146 µmol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by silica gel chromatography to give **11.10** (18 mg, 50 %).

Ethyl lactate phosphonate 11.11: A methylene chloride solution (0.8 mL) of **11.10** (18 mg, 15.8 µmol) was treated with TFA (0.2 mL) for 4 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **11.11** (6 mg, 50 %). NMR (CDCl₃ + ~10 %CD₃OD): δ 7.0-7.3 (m, 16 H), 6.8-7.0 (m, 2H), 4.9-5.0 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H). 3.5-4.0 (m, 10H), 2.18-2.3. (m, 1H), 1.6-1.7 (m, 1), 1.47 & 1.41 (2d, 3H), 1.22 (t, 3H). P NMR (CDCl₃ + ~10 %CD₃OD): 19.72 & 17.86 ppm.

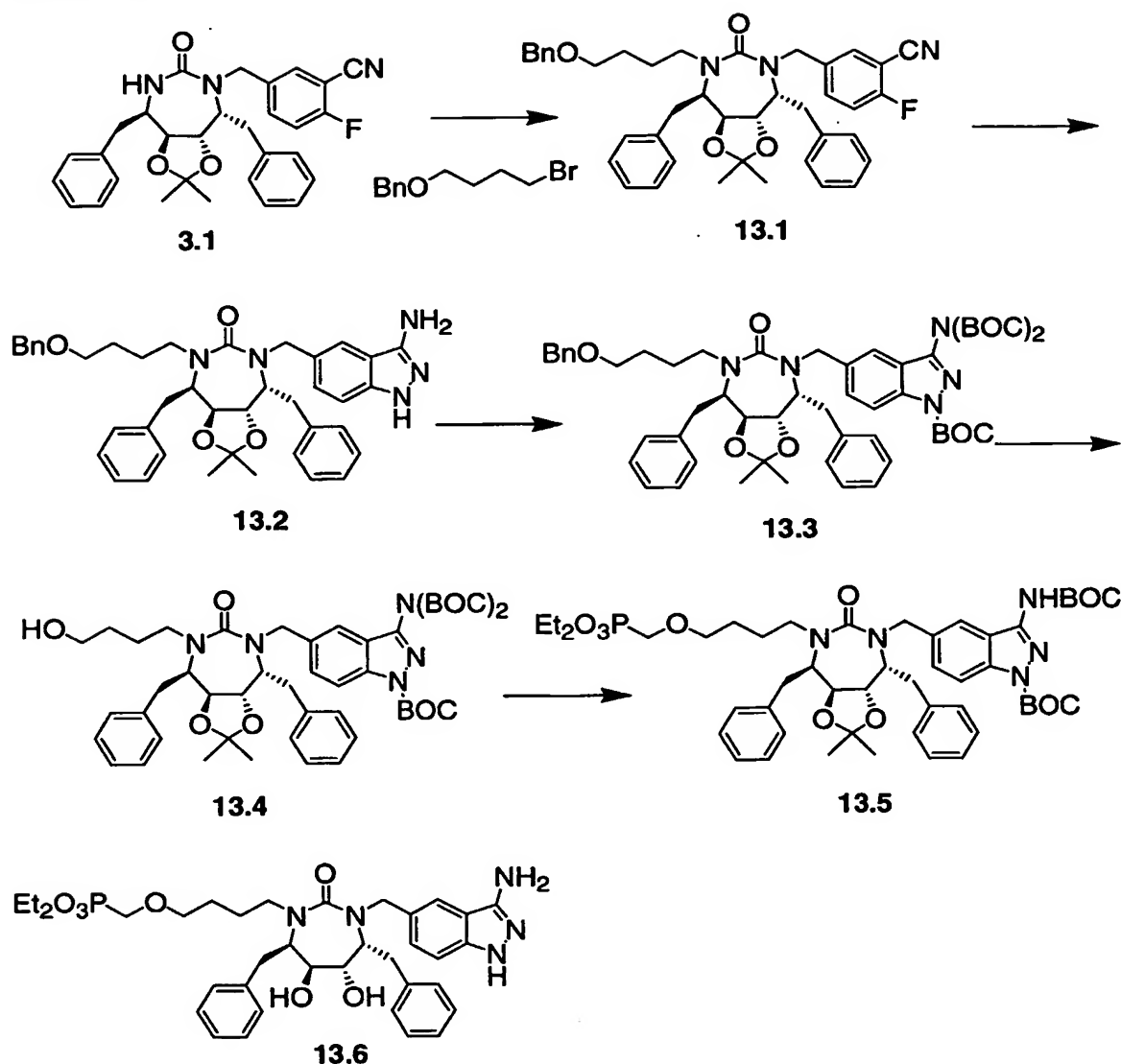
Diethyl phosphonate 11.13: Compound **11.13** (6 mg) was prepared as described above in Scheme 5 from **11.4** (30 mg, 34 µmol) and triflate phosphonate **5.3** (52 mg, 172 µmol), followed by TFA treatment. NMR (CDCl₃ + ~10 %CD₃OD): δ 7.1-7.32 (m, 11 H), 6.9-7.0 (d, 2H), 4.75 (d, 1H), 4.1-4.2 (2q, 4H), 3.84-3.9 (m, 1H), 3.4-3.8 (m, 8H), 2.7-3.1 (m, 4H), 2.1-2.5 (m, 1H), 1.5-1.7 (m, 2H), 1.25-1.35 (2t, 6H). P NMR (CDCl₃ + ~10 %CD₃OD): 21.63 ppm. MS: 680 (M + 1).

Scheme 12



- 5 **Butyl lactate phosphonate 12.2:** A pyridine solution (0.3 mL) of **11.9** (27 mg, 22 μ mol), butyl lactate (31 mg, 265 μ mol), and DCC (28 mg, 132 μ mol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by preparative TLC to give **12.1** (12 mg). A methylene chloride solution (0.8 mL) of **12.1** (12 mg) was treated with TFA (0.2 mL) for 4 h, concentrate. The residue was purified by preparative TLC to give **12.2** (3 mg, 16 %). NMR (CDCl_3 + ~10 % CD_3OD): δ 6.8-7.4 (m, 18H), 6.4-6.6 (m), 4.9-5.05 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H). 3.5-4.0 (m, 10H), 3.1-3.25 (m, 2H), 2.2-2.35 (m, 1H), 1.8-1.9 (m, 1H), 1.4 & 1.8 (m, 7H), 1.22 (t, 3H). P NMR (CDCl_3 + ~10 % CD_3OD): 19.69 & 17.86 ppm.
- 10

Scheme 13



Benzyl ether 13.1: A DMF solution (5 mL) of **3.1** (1 g, 2 mmol) was treated with NaH (0.24 g of 60% oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol), and benzyloxybutyl bromide (0.58 g, 2.4 mmol). After the reaction for 5 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **13.1** (0.58 g, 44 %).

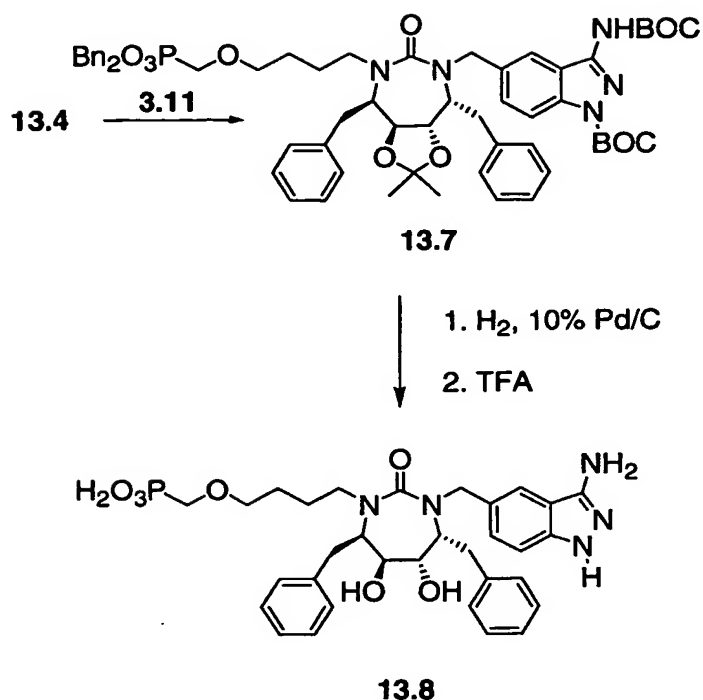
Aminoindazole 13.2: A n-butanol solution (10 mL) of **11.1** (0.58 g, 0.87 mmol) and hydrazine hydrate (0.88 g, 17.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude **13.2** (0.56 g).

Tri-BOC-aminoindazole 13.3: A methylene chloride solution (10 mL) of **13.2** (0.55 g, 0.82 mmol), DIPEA (0.42 g, 3.2 mmol), (BOC)₂O (0.71 g, 3.2 mmol), and DMAP (0.3 g, 2.4 mmol) was stirred for 4 h at room temperature, partitioned between methylene chloride and 5% citric acid solution, dried, purified by silica gel chromatography to give **13.3** (0.56 g, 71 %, 2 steps).

3-Hydroxybutyl cyclic urea 13.4: An ethyl acetate/methanol solution (30 mL/5 mL) of **11.3** (0.55 g, 0.56 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (0.2 g) for 3 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford crude **13.4** (0.5 g, 98 %).

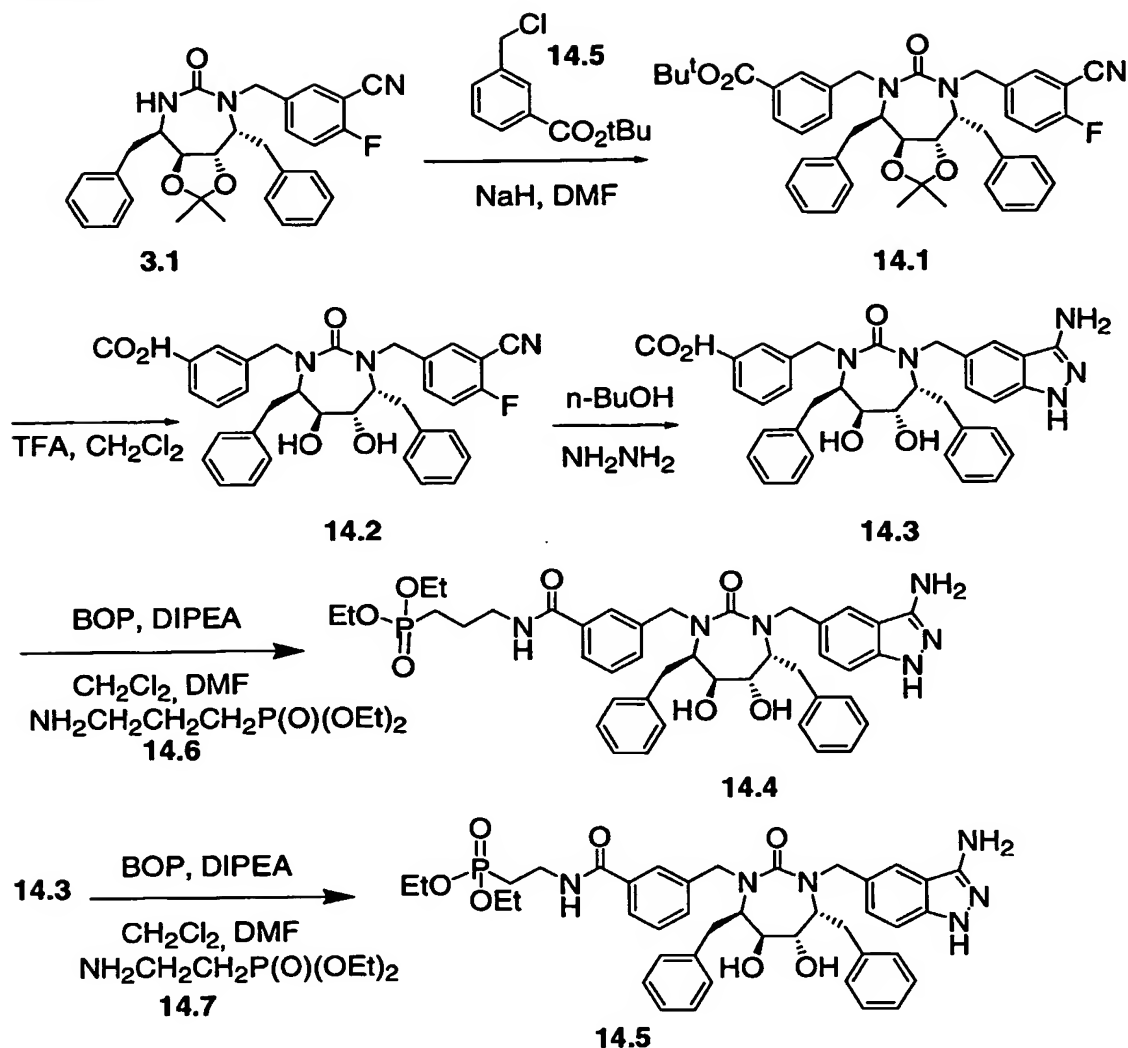
Diethyl phosphonate 13.6: A THF solution (1 mL) of **13.4** (5 mg, 56 μ mol) and triflate diethyl phosphonate **5.3** (30 mg, 100 μ mol) was cooled to -3°C , followed by addition of n-BuLi (80 μ l of 2.5 M hexane solution, 200 μ mol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure to give crude **13.5**. The residue was dissolved in methylene chloride (0.8 mL) and treated with TFA (0.2 mL) for 4 h. concentrated under reduced pressure, and purified by HPLC to give **13.6** (8 mg, 21%). NMR (CDCl₃): δ 7.1-7.4 (m, 11H), 7.0-7.1 (m, 2H) 4.81 (d, 1H), 4.1-4.25 (m, 4H). 3.85-3.95 (m, 1H), 3.4-3.8 (m, 7H), 3.3-3.4 (m, 1H), 2.8 - 3.25 (m, 5H), 2.0-2.15 (m, 1H), 1.3-1.85 (m, 10H). P NMR (CDCl₃): 21.45 ppm.

Scheme 13a



- 5 Phosphonic diacid 13.8:** Compound **13.8** (4.5 mg) was prepared from **13.4** as described above for the preparation of **11.7** from **11.4** (Scheme 11). NMR (CD_3OD): δ 7.41 (s, 1H), 7.1-7.4 (m, 10H), 6.9-7.0 (m, 2H) 4.75 (d, 1H), 3.8-4.0 (m, 1H). 3.4-3.8 (m, 8H), 2.8-3.25 (m, 5H), 2.1-2.25 (m, 1H), 1.6-1.85 (m, 4H). MS: 638 ($\text{M} + 1$).

Scheme 14



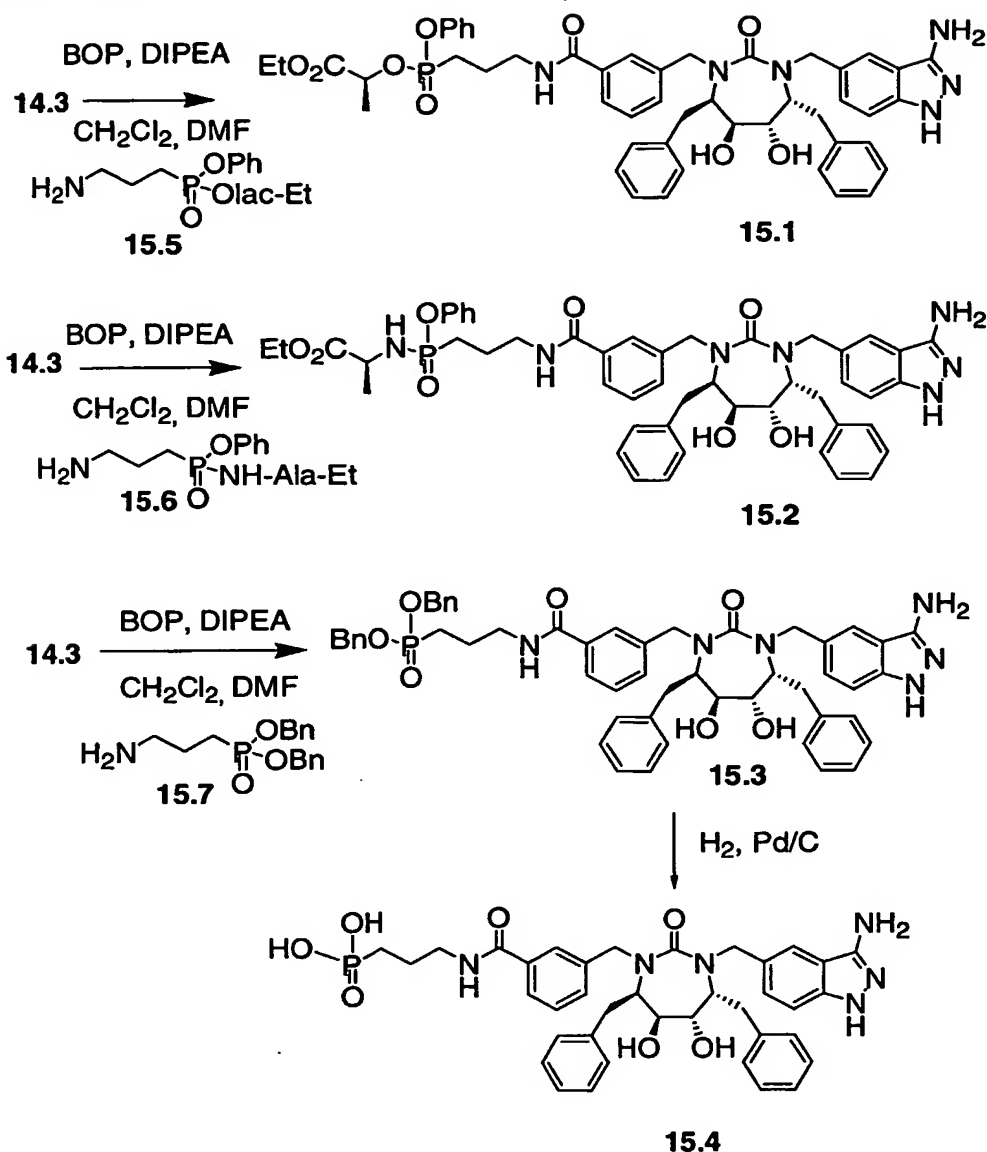
- 5 **t-Butyl ester 14.1:** A DMF solution (3 mL) of **3.1** (0.5 g, 1 mmol) was treated with NaH (80 mg of 60% oil dispersion, 2 mmol) for 10 min, followed by the addition of **14.5** (0.25 g, 1.1 mmol). After the reaction for 1 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **14.1** (0.4 g, 59%).
- 10 **Aminoindazole derivative 14.3:** A methylene chloride solution (5 mL) of **14.1** (0.4 g, 0.58 mmol) was treated with TFA (1 mL) at room temperature for 1.5 h, and then concentrated under reduced pressure to give crude **14.2**. The crude **14.2** was dissolved in n-BuOH (5 mL) and reacted with hydrazine hydrate (0.58 g, 11.6 mmol) at reflux for 5 h. The reaction

mixture was concentrated under reduced pressure and purified by silica gel chromatography to give the desired product **14.3** (0.37 g, quantitative yield).

Diethylphosphonate ester 14.4: A methylene chloride solution (3 mL) of **14.3** (23 mg, 38 μmol) was reacted with aminopropyl-diethylphosphonate **14.6** (58 mg, 190 μmol), DIPEA (50 mg, 380 μmol), and ByBOP (21 mg, 48 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **14.4** (9 mg, 34 %). NMR (CDCl_3 + ~10 % CD_3O): δ 7.87 (t, 1H), 7.61 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 10 H), 6.93-7.0 (m, 4H), 4.79 (d, 2H), 3.99-4.04 (m, 4H), 3.38-3.65 (m, 6H), 2.60-3.2 (m, 6 H), 1.70-1.87 (m, 4H), 1.25 (t, 6H). P NMR (CDCl_3 + ~10 % CD_3OD): 32.7 ppm.

Diethylphosphonate ester 14.5: A methylene chloride solution (2 mL) of **14.3** (13 mg, 21 μmol) was reacted with aminoethyl-diethylphosphonate oxalate **14.7** (23mg, 85 μmol), DIPEA (22 mg, 170 μmol), and ByBOP (12 mg, 25 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **14.5** (5mg, 30%). Ms: 783 (M + 1). NMR (CDCl_3 + ~10 % CD_3O): δ 7.88 (b, 1H), 7.58 (b, 1H), 7.49 (s, 1H), 7.14-7.2 (m, 10 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.90-4.04 (m, 4H), 2.50-3.3 (m, 6 H), 1.97-2.08 (m, 2H). P NMR (CDCl_3 + ~10 % CD_3OD): 30.12 ppm.

Scheme 15



Monophenol-ethyl lactate phosphonate prodrug 15.1: A methylene chloride/DMF

- 5 solution (2 mL/0.5 mL) of 14.3 (30 mg, 49 μ mol) was reacted with aminopropyl-phenol-ethyl lactate phosphonate 15.5 (100 mg, 233 μ mol), DIPEA (64 mg, 495 μ mol), and BOP reagent (45 mg, 100 μ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by silica gel chromatography to give 15.1 (28 mg, 64 %). NMR (CDCl₃ + ~10 %CD₃O): δ
- 10 7.83 (b, 1H), 7.59 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75-4.87 (d +

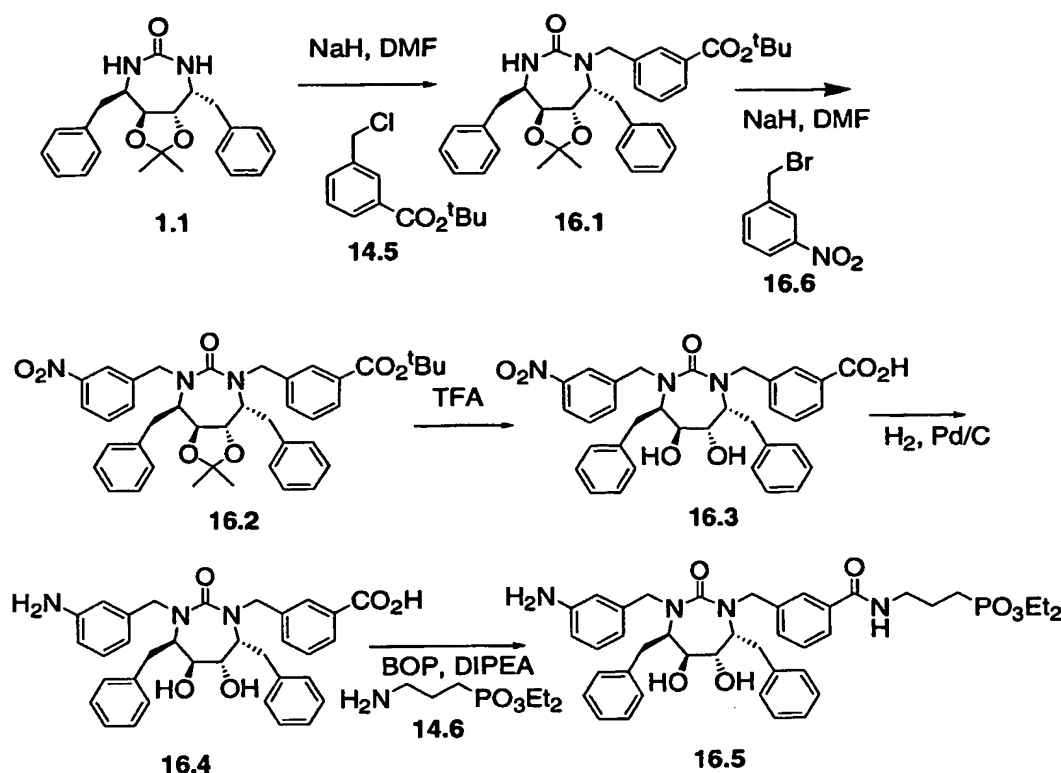
q, 3H), 4.10 (q, 2H), 3.3-3.61 (m, 6H), 2.60-3.2 (m, 6H), 1.92-2.12 (m, 4H), 1.30 (d, 3H), 1.18 (t, 3H). P NMR (CDCl_3 + ~10 % CD_3OD): 30.71 ppm. MS: 903 (M + 1).

Phenol-ethyl alanine phosphonate prodrug 15.2: A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49 μmol) was reacted with aminopropyl-phenol-ethyl alanine phosphonate **15.6** (80 mg TFA salt, 186 μmol), DIPEA (64 mg, 500 μmol), and BOP reagent (45 mg, 100 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.2** (12 mg, 27 %). NMR (CDCl_3 + ~10 % CD_3O): δ 7.91 (b, 1H), 7.61 (b, 1H), 7.52 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.82-4.1 (2q, 3H), 3.4-3.65 (m, 6H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H), 1.3 (d, 3H). P NMR (CDCl_3 + ~10 % CD_3OD): 32.98 & 33.38 ppm. MS: 902 (M + 1).

Dibenzyl phosphonate 15.3: A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49 μmol) was reacted with aminopropyl dibenzyl phosphonate **15.7** (86 mg TFA salt, 200 μmol), DIPEA (64 mg, 500 μmol), and BOP reagent (45 mg, 100 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.3** (20 mg, 44%). NMR (CDCl_3 + ~5% CD_3O): δ 7.50-7.58 (m, 2H), 7.14-7.3 (m, 21 H), 6.90-7.0 (m, 4H), 4.7-5.1 (m, 6H), 3.6-3.8 (m, 4H), 3.3-3.55 (m, 2H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H). P NMR (CDCl_3 + ~5 % CD_3OD): 33.7 ppm. MS: 907 (M + 1).

Phosphonic diacid 15.4: An ethanol solution (5 mL) of **15.3** (17 mg, 18.7 μmol) was hydrogenated at 1 atm in the presence of 10 % Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **15.4** (12 mg, 85%). NMR (CD_3O + 20% CDCl_3): δ 7.88 (b, 1H), 7.59 (b, 1H), 7.6 (s, 1H), 7.1-7.25 (m, 10 H), 6.90-7.1 (m, 4H), 4.8 (d, 2H + water peak), 3.6-3.8 (m, 4H), 3.4-3.5 (m, 2H), 1.85-2.0 (m, 4H).

Scheme 16



Monobenzylic derivative 16.1: A DMF solution (4 mL) of 1.1 (0.8 g, 2.2 mmol) was treated with NaH (0.18 g of 60% oil dispersion, 4.4 mmol) for 10 min at room temperature followed by the addition of 14.5 (0.5 g, 2.2 mmol). The resulting solution was reacted at room temperature for 2 h, worked up, and then purified to afford 16.1 (0.48 g, 40%).

3-Nitrobenzyl cyclic urea derivative 16.2: A DMF solution (0.5 mL) of 16.1 (65 mg, 117 μ mol) was treated with NaH (15 mg of 60% oil dispersion, 375 μ mol) for 10 min at room temperature, followed by the addition of 3-nitrobenzyl bromide (33 mg, 152 μ mol). The resulting solution was reacted at room temperature for 1 h, worked up, and purified by preparative TLC to afford 16.2 (66 mg, 82%).

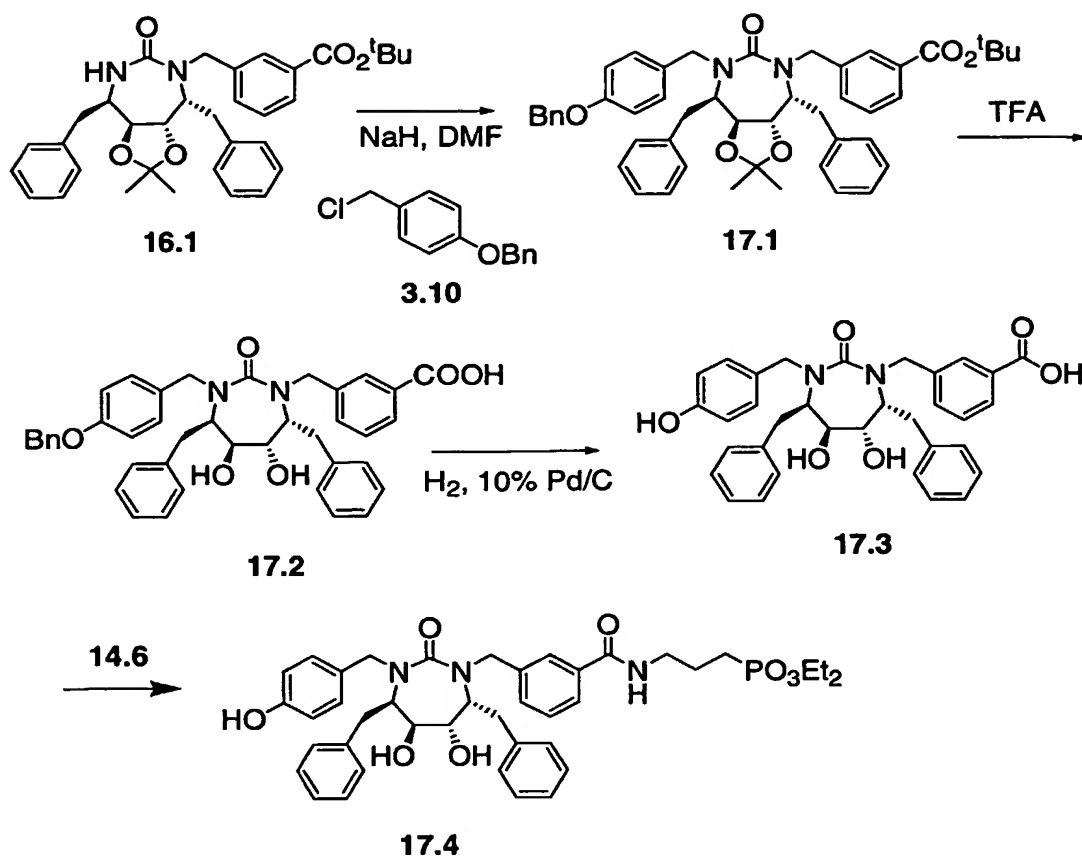
Diol 16.3: A methylene chloride solution (2 mL) of 16.2 (46 mg, 61 μ mol) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to afford 16.3. This material was used without further purification.

3-Aminobenzyl cyclic urea 16.4: An ethyl acetate/ethanol (5 mL/1 mL) solution of **16.3** (crude) was hydrogenated at 1 atm in the presence of 10% Pd/C for 2 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to afford **16.4** (26 mg, 70%, 2 steps).

5

Diethyl phosphonate 16.5: A methylene chloride/DMF solution (2 mL/0.5 mL) of **16.4** (24 mg, 42 μ mol) was reacted with aminopropyl-diethylphosphonate ester TFA salt **14.6** (39 mg, 127 μ mol), DIPEA (27 mg, 210 μ mol), and BOP reagent (28 mg, 63 μ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified
10 by preparative TLC to give **16.5** (20.7 mg, 63 %). NMR (CDCl_3 + ~10 % CD_3O): δ 7.62 (b, 1H), 7.51 (s, 1H), 7.0-7.35 (m, 12 H), 6.95 (d, 2H), 6.85 (d, 2H), 4.6-4.71 (2d, 2H), 3.95-4.1 (m, 4H). 3.3-3.55 (m, 3H), 2.60-2.8 (m, 2H), 2.95-3.15 (m, 4 H), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR (CDCl_3 + ~10 % CD_3OD): 32.65 ppm.

Scheme 17



p-Benzoybenzyl cyclic urea derivative 17.1: A DMF solution (0.5 mL) of 16.1 (65 mg, 117 μ mol) was treated with NaH (15 mg of 60% oil dispersion, 375 μ mol) for 10 min at room temperature, followed by the addition of 4-benzyloxy benzyl chloride 3.10 (35 mg, μ mol). The resulting solution was stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure, purified by preparative TLC to generate 17.1 (62 mg, 70%).

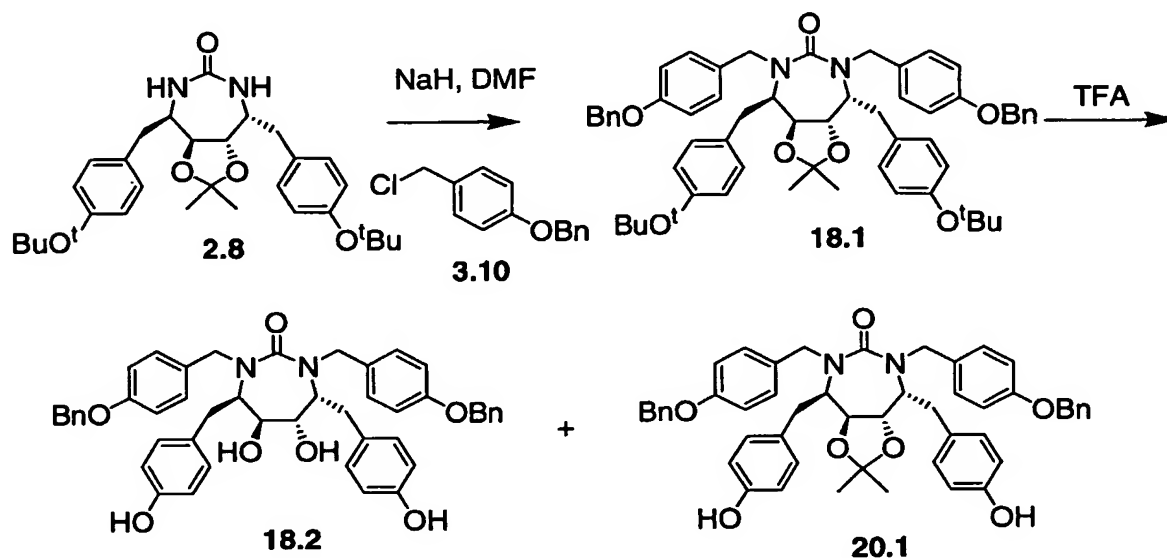
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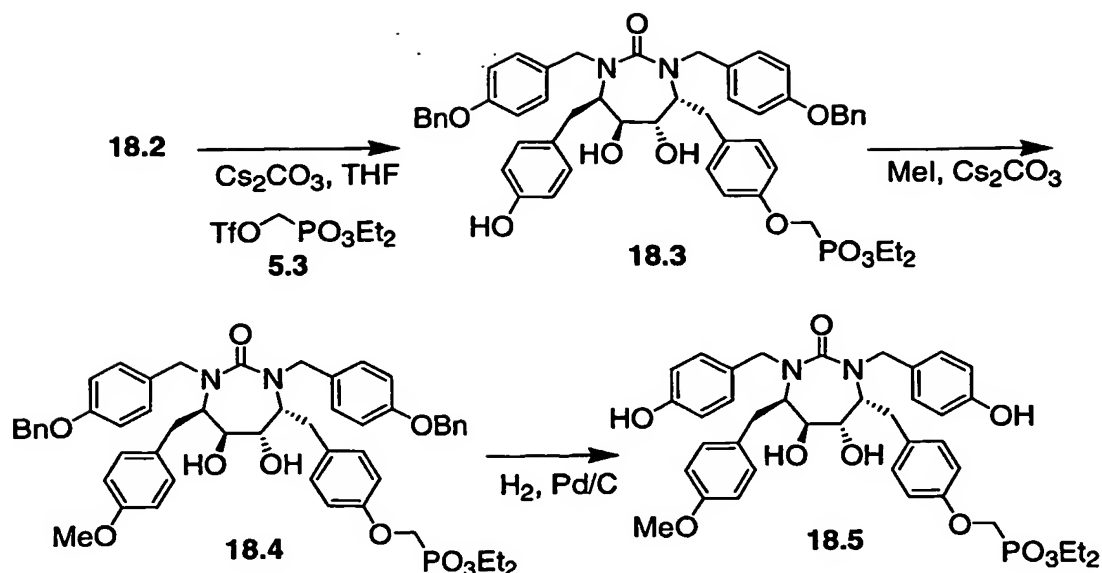
Diethyl phosphonate 17.3: A methylene chloride solution (2 mL) of 17.1 (46 mg, 61 μ mol) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to give crude 17.2. An ethyl acetate/ethanol solution (3 mL/2 mL) of the crude 17.2 was then hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 5 h at room temperature. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford 17.3 (crude).

15

Diethyl phosphonate cyclic urea 17.4: A methylene chloride/DMF solution (2 mL/0.5 mL) of **17.3** (25 mg, 42 μ mol) was reacted with aminopropyl-diethylphosphonate ester TFA salt **14.6** (40 mg, 127 μ mol), DIPEA (27 mg, 210 μ mol), and BOP reagent (28 mg, 63 μ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **17.4** (14.6 mg, 44 %). NMR ($\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$): δ 7.82 (t), 7.62 (d, 1H), 7.51 (s, 1H), 7.05-7.35 (m, 10 H), 6.8-6.95 (2d, 4H), 6.85 (d, 2H), 4.8 (d, 1H), 4.65 (d, 1H), 3.95-4.1 (m, 4H), 3.4-3.75 (m, 6H), 2.60-3.2 (m), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR ($\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$): 32.72 ppm.

10 Scheme 18





Dibenzyl derivative 18.1: A DMF solution (3 mL) of compound 2.8 (0.4 g, 0.78 mmol) was reacted with 60%NaH (0.13 g, 1.96 mmol), 4-benzoxyl benzylchloride 3.10 (0.46 g, 1.96 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 4 h. The reaction mixture was partitioned between methylene chloride and saturated NaHCO₃ solution. The organic phase was isolated, dried over Na₂SO₄, concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product 18.1 (0.57 g, 81%).

Diol derivative 18.2 and diphenol derivative 20.1: A methylene chloride solution (4 mL) of 18.1 (0.57 g, 0.63 mmol) was treated with TFA (1 mL) at room temperature for 20 min, concentrated under reduced pressure, and purified by silica gel chromatography to give diol derivative 18.2 (133 mg, 28 %) and diphenol derivative 20.1 (288 mg, 57.6%).

Monophosphonate derivative 18.3: A THF solution (10 mL) of 18.2 (130 mg, 0.17 mmol) was stirred with cesium carbonate (70 mg, 0.21 mmol) and diethylphosphonate triflate 5.3 (52 mg, 0.17 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give 18.3 (64 mg, 41 %), and recovered 18.2 (25 mg, 19%).

Methoxy derivative 18.4: A THF solution (2 mL) of 18.3 (28 mg, 25 μmol) was treated with cesium carbonate (25 mg, 76 μmol) and iodomethane (10 eq. Excess) at room

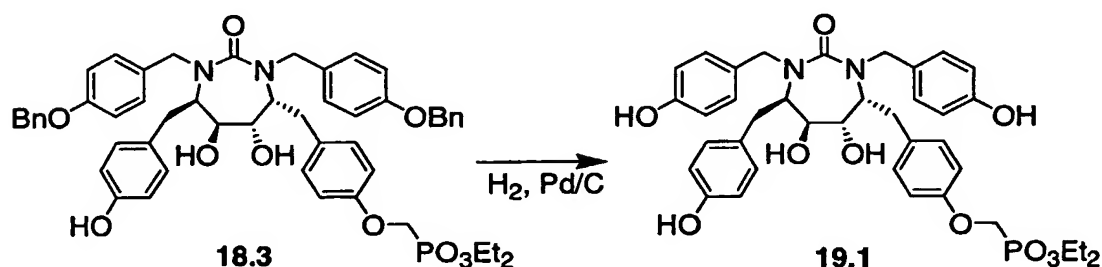
temperature for 5 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated NaHCO₃. The organic phase was separated, concentrated under reduced pressure and the residue purified by preparative TLC to afford **18.4** (22 mg, 78%).

5

Diethylphosphonate 18.5 : An ethyl acetate/ethanol (2 mL/2 mL) solution of **18.4** (22 mg, 24 μ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C for 3 h. The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure to give the desired product **18.5** (18 mg, quantitative). NMR (CDCl₃ + ~10 %CD₃O): δ 6.7-7.0 (m, 12 H), 6.62-6.69 (m, 4H), 4.65 (d, 1H), 4.50 (d, 1H), 4.18-4.3 (m, 6H). 3.75 (s, 3H), 3.3-3.4 (m, 4H), 2.8-3.0 (m, 6H), 1.30 (t, 6H). P NMR (CDCl₃ + ~10 %CD₃OD): 20.16 ppm.

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Scheme 19

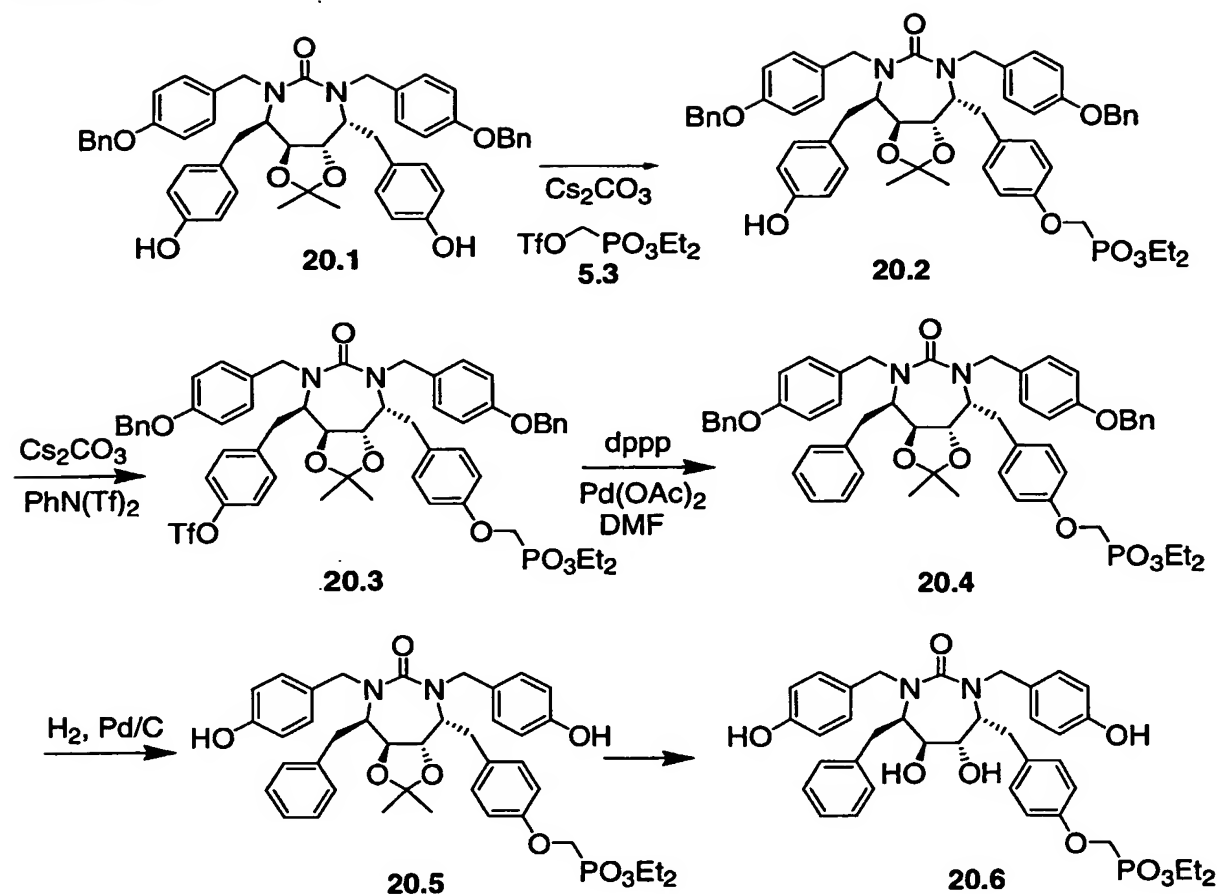


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Diethyl phosphonate 19.1: An ethyl acetate/ethanol (2 mL/1 mL) solution of **18.3** (14 mg, 15.5 μ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **19.1** (10 mg, 90%). NMR (CDCl₃ + ~15 %CD₃O): δ 6.6-7.0 (m, 16 H), 4.5-4.65 (2d, 2H), 4.1-4.3 (m, 6H). 2.7-3.0 (m, 6H), 1.29 (t, 6H). P NMR (CDCl₃ + ~15 %CD₃OD): 20.12 ppm.

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Scheme 20

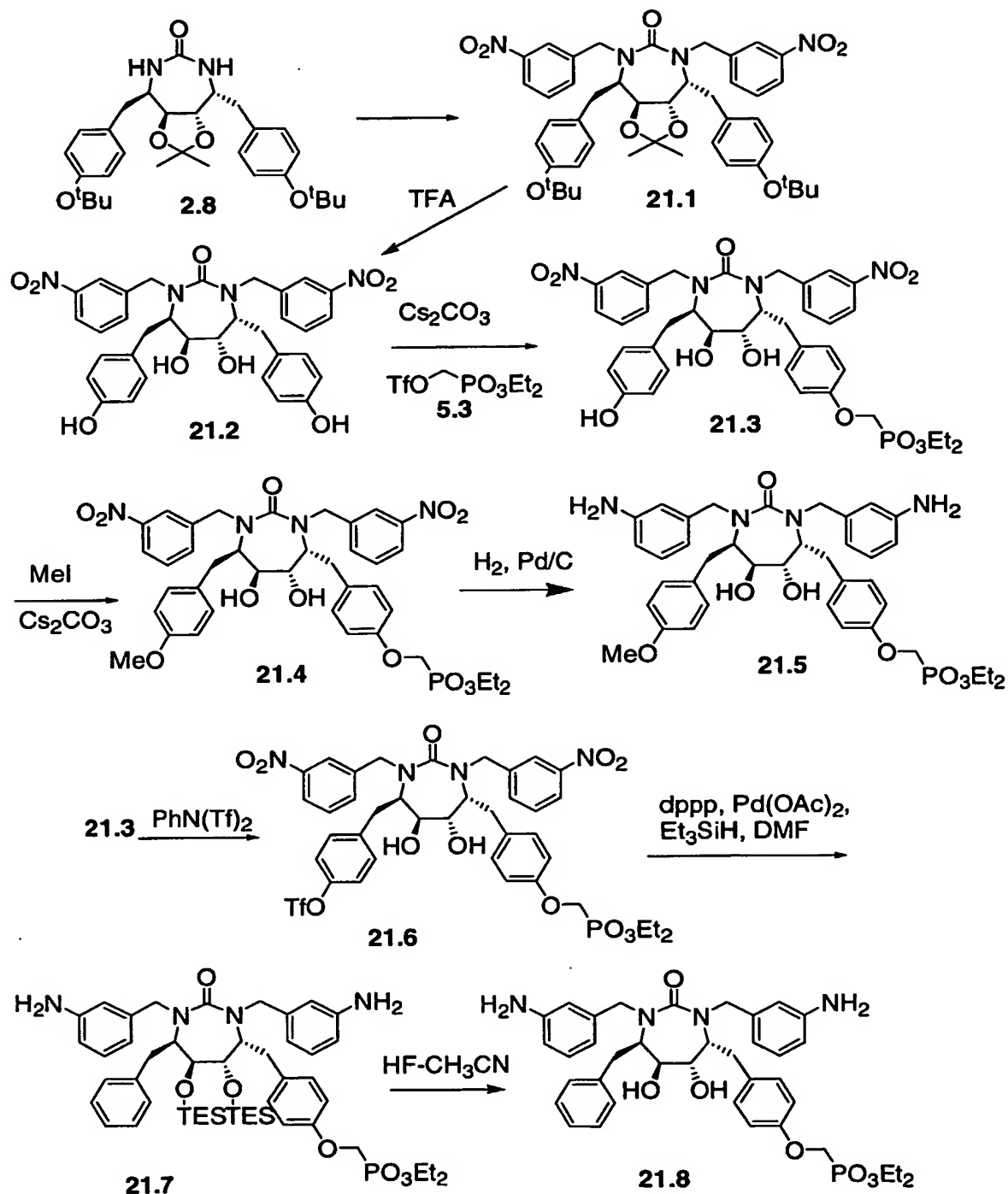


- 5 **Monophosphonate 20.2:** A THF solution (8 mL) of 20.1 (280 mg, 0.36 mmol) was stirred with cesium carbonate (140 mg, 0.43 mmol) and diethylphosphonate triflate 5.3 (110 mg, 0.36 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give 20.2 (130mg, 39%), and recovered 20.1 (76 mg, 27%).
- 10 **Triflate derivative 20.3:** A THF solution (6 mL) of 20.2 (130 mg, 0.13 mmol) was stirred with cesium carbonate (67 mg, 0.21 mmol) and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give 20.3 (125 mg, 84%).
- 15 **Benzyl ether 20.4:** To a DMF solution (2 mL) of $\text{Pd}(\text{OAc})_2$ (60 mg, 267 μmol), and dppp (105 mg, 254 μmol) was added 20.3 (120 mg, 111 μmol) under nitrogen, followed by the addition of triethylsilane (0.3 mL). The resulting solution was stirred at room temperature for

4 h, then concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford **20.4** (94 mg, 92%).

Diethyl phosphonate 20.6: An ethyl acetate/ethanol (2 mL/2 mL) solution of **20.4** (28 mg, 30 μ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **20.5**. The crude product **20.5** was redissolved in methylene chloride (2 mL) and treated with TFA (0.4 mL) and a drop of water. After 1 h stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and
5
10 purified by preparative TLC plate to give **20.6** (18 mg, 85 %, 2 steps). δ 6.6-7.3 (m, 17 H), 4.65 (d, 1H), 4.58 (d, 1H), 4.18-4.3 (m, 6H), 3.3-3.5 (m, 4H), 2.8-3.1 (m), 1.34 (t, 6H). P NMR (CDCl₃ + ~10 %CD₃OD): 20.16 ppm. MS: 705 (M + 1).

Scheme 21



Bis-(3-nitrobenzyl) derivative 21.1: A DMF solution (2 mL) of compound 2.8 (0.3 g, 0.59 mmol) was reacted with 60%NaH (0.07 g, 1.76 mmol), 3-nitrobenzyl bromide (0.38 g, 1.76 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 3 h. The reaction

mixture was partitioned between methylene chloride and saturated NaHCO_3 solution. The organic phase was isolated, dried over Na_2SO_4 , concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product **21.1** (0.37 g, 82%).

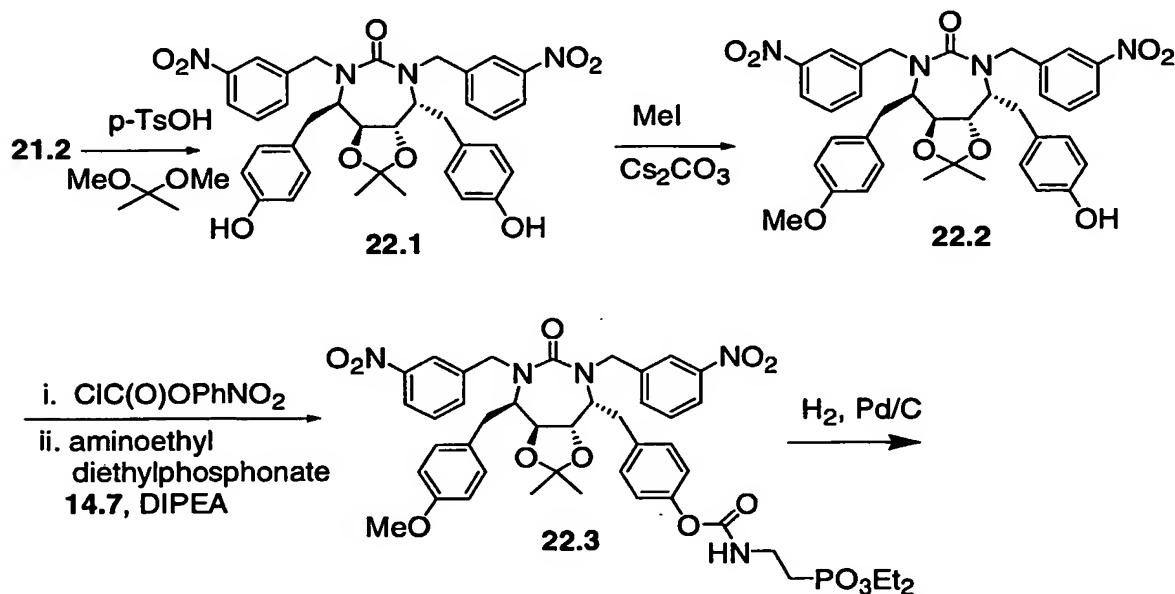
- 5 **Diphenol derivative 21.2:** A methylene chloride solution (4 mL) of **21.1** (0.37 g, 0.47 mmol) was treated with TFA (1 mL) at room temperature for 3 h, and then concentrated under reduced pressure, and azeotroped with CH_3CN twice to give diphenol derivative **21.2** (0.3 g, quantitative).
- 10 **Monophosphonate derivative 21.3:** A THF solution (8 mL) of **18.2** (0.28g, 0.44 mmol) was stirred with cesium carbonate (0.17 g, 0.53 mmol) and diethylphosphonate triflate **5.3** (0.14 g, 0.44 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give **21.3** (120 mg, 35%), and recovered **21.2** (150 mg, 53%).
- 15 **Methoxy derivative 21.4:** A THF solution (2 mL) of **21.3** (9 mg, 11 μmol) was treated with cesium carbonate (15 mg, 46 μmol) and iodomethane (10 eq. Excess) at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated NaHCO_3 . The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by preparative TLC to afford **21.4** (9 mg)
- 20 **Diethylphosphonate 21.5:** A ethyl acetate/ethanol (2 mL/0.5 mL) solution of **21.4** (9 mg, 11 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **21.5** (4.3 mg, 49%, 2 steps). NMR (CDCl_3 + ~10 % CD_3O): δ 7.0-7.10 (m, 6 H), 6.8-6.95 (m, 4H), 6.5-6.6 (m, 4H), 6.4-6.45 (m, 2H), 4.72 (d, 2H), 4.18-4.3 (m, 6H). 3.72 (s, 3H), 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR (CDCl_3 + ~10 % CD_3OD): 19.93 ppm.
- 25 **Triflate 21.6:** A THF solution (6 mL) of **21.3** (0.1g, 0.14 mmol), cesium carbonate (0.07 g, 0.21 mmol), and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) was stirred at
- 30

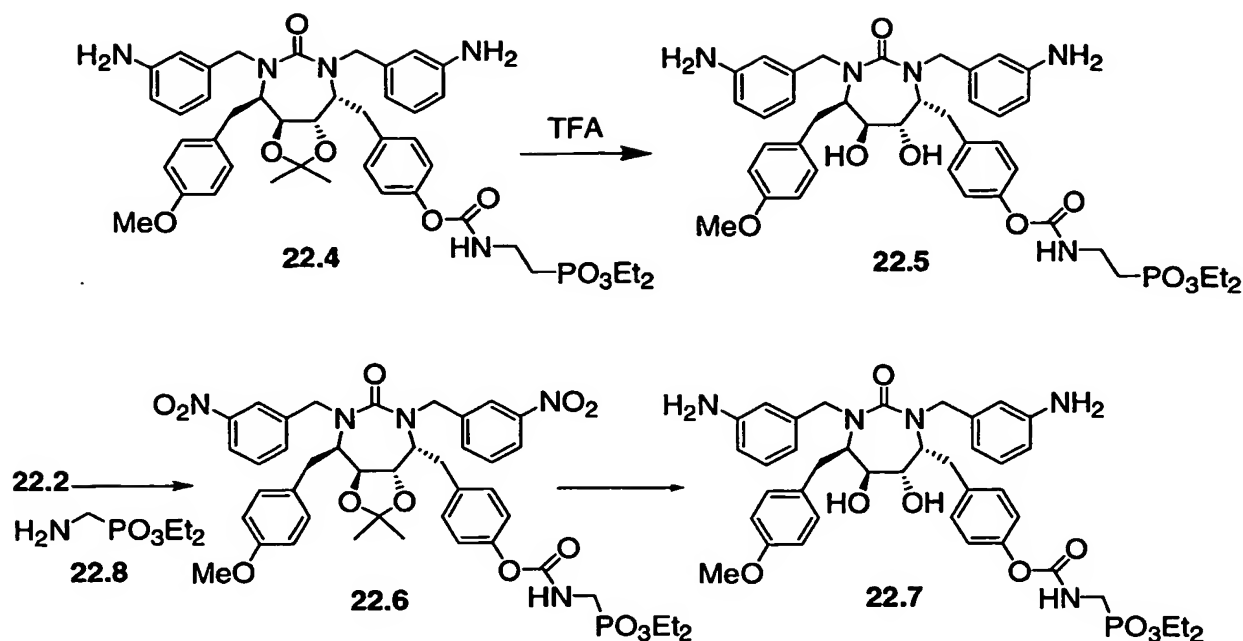
room temperature for 4 h, and then concentrated under reduced pressure, and worked up. The residue was purified by silica gel chromatography to give **21.6** (116 mg, 90%).

Diamine 21.7: A DMF solution (2 mL) of **21.6** (116 mg, 127 μmol), dppp (60 mg, 145 μmol), and $\text{Pd}(\text{OAc})_2$ (30 mg, 134 μmol) was stirred under nitrogen, followed by addition of triethylsilane (0.3 mL), and reacted for 4 h at room temperature. The reaction mixture was worked up and purified to give **21.7** (50 mg).

Diethyl phosphonate 21.8: An acetonitrile solution (1 mL) of crude **21.7** (50 mg) was treated with 48% HF (0.1 mL) for 4 h. The reaction mixture was concentrated under reduced pressure, and purified to give **21.8** (10 mg, 11% (2 steps). NMR ($\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$): δ 7.05-7.30 (m, 9 H), 6.8-6.95 (d, 2H), 6.4-6.6 (m, 6H), 4.72 (d, 2H), 4.18-4.3 (m, 6H). 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR ($\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$): 19.83 ppm.

15 Scheme 22





Acetonide 22.1: An acetone/2,2-dimethoxypropane solution (15 mL/5 mL) of compound 21.2 (240 mg, 0.38 mmol) and pyridinium toluenesulfonate (10 mg) was heated at reflux for 30 min. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between methylene chloride and saturated NaHCO₃ aqueous solution, dried, concentrated under reduced pressure and purified to afford 22.1 (225 mg, 88%).

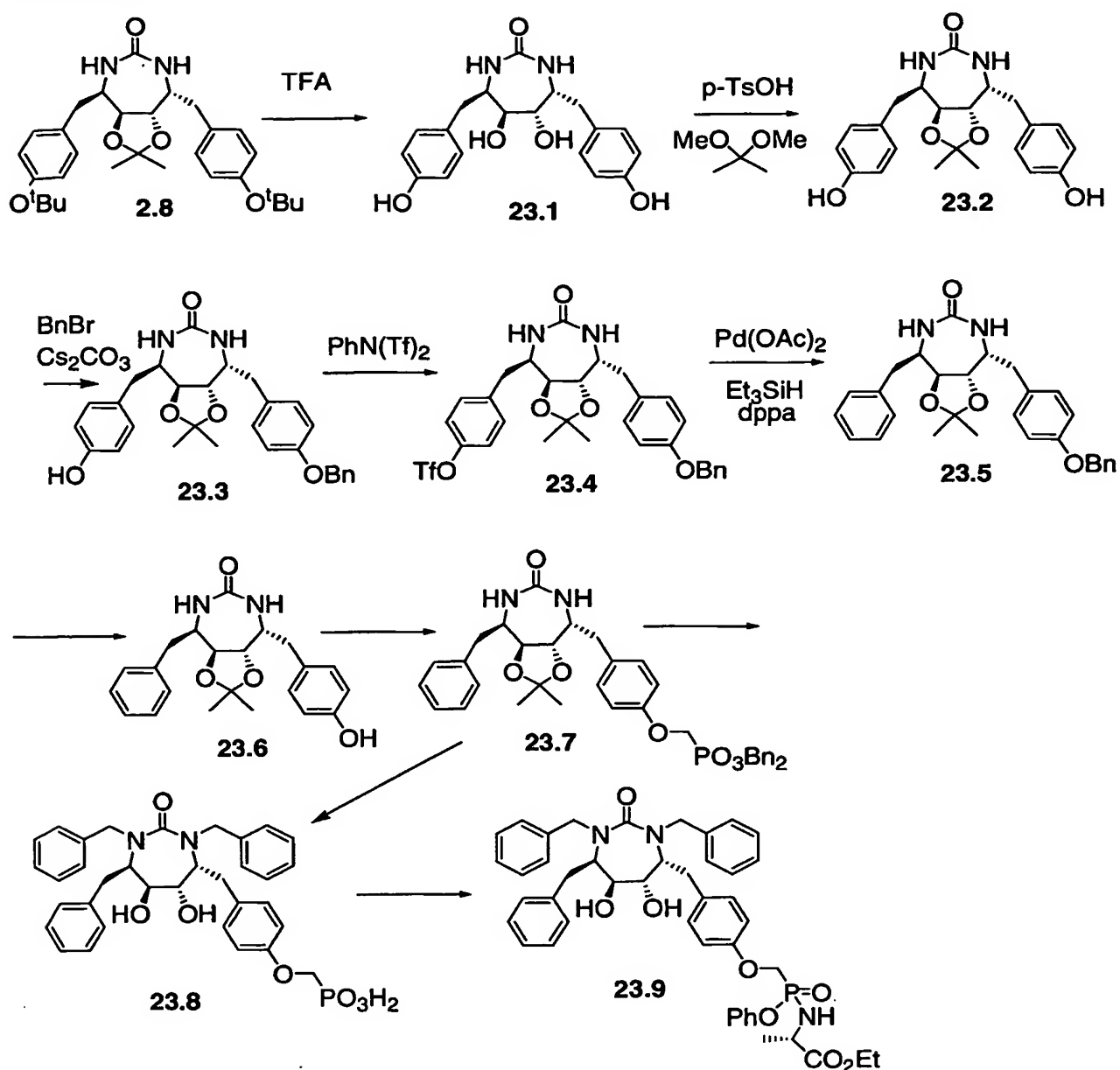
Monomethoxy derivative 22.2: A THF solution (10 mL) of 22.1 (225 mg, 0.33 mmol) was treated with cesium carbonate (160 mg, 0.5 mmol) and iodomethane (52 mg, 0.37 mmol) at room temperature overnight. The reaction mixture was concentrated under reduced pressure, and purified by preparative silica gel column chromatography to afford 22.2 (66 mg, 29%) and recovered starting material 22.1 (25 mg, 11%).

Diethyl phosphonate 22.3: A methylene chloride solution (2 mL) of 22.2 (22 mg, 32 μmol), DIPEA (9 mg, 66 μmol), and p-nitrophenyl chloroformate (8 mg, 40 μmol) was stirred at room temperature for 30 min. The resulting reaction mixture was reacted with DIPEA (10 mg, 77 μmol), and aminoethyl diethylphosphonate 14.7 (12 mg, 45 μmol) at room temperature overnight. The reaction mixture was washed with 5% citric acid solution, saturated NaHCO₃, dried, and purified by preparative TLC to afford 22.3 (12 mg, 43%).

Bis(3-aminobenzyl)-diethylphosphonate ester 22.5: An ethyl acetate/t-BuOH (4 mL/2 mL) solution of **22.3** (12 mg, 13 μ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C 95 mg) at room temperature for 5 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to give **22.4** (8 mg, 72%). A methylene chloride solution (0.5 mL) of **22.4** (8 mg) was treated with TFA (0.1 mL) at room temperature for 1 h., concentrated under reduced pressure, and then azeotroped with CH₃CN twice to afford **22.5** (8.1 mg, 81%). NMR (CDCl₃ + ~10 %CD₃OD): δ 7.2 (d, 1H), 6.95-7.15 (m, 6H), 6.75-6.9 (m, 5 H), 4.66 (d, 1H), 4.46 (d, 1H), 4.06-4.15 (m, 4H). 3.75 (s, 3H), 3.6-3.7 (m, 4H), 2.6-3.1 (m, 6H), 2.0-2.1 (m, 2H), 1.30 (t, 6H). P NMR (CDCl₃ + ~10 %CD₃OD): 29.53 ppm. MS: 790 (M + 1).

Bis(3-aminobenzyl) diethylphosphonate ester 22.7: Compound **22.7** was prepared from **22.2** (22 mg, 32 μ mol) and aminomethyl diethylphosphonate **22.8** as shown above for the preparation of **22.5** from **22.2**. NMR (CDCl₃ + ~10 %CD₃OD): δ 7.24 (d, 1H), 6.8-7.12 (m, 11H), 4.66 (d, 1H), 4.45 (d, 1H), 4.06-4.15 (m, 4H). 3.75 (s, 3H), 2.6-3.1 (m, 6H), 1.30 (t, 6H). P NMR (CDCl₃ + ~10 %CD₃OD): 22.75 ppm. MS: 776 (M + 1).

Scheme 23



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Diol 23.1: To a solution of compound **2.8** (2.98 g, 5.84 mmol) in methylene chloride (14 mL) was added TFA (6 mL). The resulted mixture was stirred at room temperature for 2 h. Methanol (5 mL) and additional TFA (5 mL) were added. The reaction mixture was stirred for additional 4 h and then concentrated under reduced pressure. The residue was washed with hexane/ethyl acetate (1:1) and dried to afford compound **23.1** (1.8 g, 86%) as an off-white solid.

Benzyl ether 23.3: To a solution of compound **23.1** (1.8 g, 5.03 mmol) in DMF (6 mL) and 2,2-dimethoxyl propane (12 mL) was added p-toluenesulfonic acid monohydrate (0.095 g, 0.5 mmol). The resultant mixture was stirred at 65°C for 3 h. The excess 2,2-dimethoxyl propane was slowly distilled. The reaction mixture was cooled to room temperature and
5 charged with THF (50 mL), benzyl bromide (0.8 mL, 6.73 mmol) and cesium carbonate (2.0 g, 6.13 mmol). The resulted mixture was stirred at 65°C for 16 h. The reaction was quenched with acetic acid aqueous solution (4%, 100 mL) at 0°C, and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired
10 mono protected compound **23.3** (1.21 g, 49%).

Benzyl ether 23.5: To a solution of compound **23.3** (0.65 g, 1.33 mmol) and N-phenyltrifluoromethanesulfonimide (0.715 g, 2 mmol) in THF (12 mL) was added cesium carbonate (0.65 g, 2 mmol). The mixture was stirred at room temperature for 3 h. The
15 reaction mixture was filtered through a pad of silica gel and concentrated under reduced pressure. The residue was purified on silica gel chromatography to give triflate **23.4** (0.85 g). To a solution of 1,3-bis(diphenylphosphino)propane (0.275g, 0.66 mmol) in DMF (10 mL) was added palladium(II) acetate (0.15 g, 0.66 mmol) under argon. This mixture was stirred for 2 min. and then added to triflate **23.4**. After stirring for 2 min., triethylsilane was added
20 and the resulted mixture was stirred for 1.5 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel to afford compound **23.5** (0.56 g, 89%).

Phenol 23.6: A solution of **23.5** (0.28 g, 0.593 mmol) in ethyl acetate (5 mL) and isopropyl
25 alcohol (5 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **23.6** (0.22 g, 97%) as a white solid.

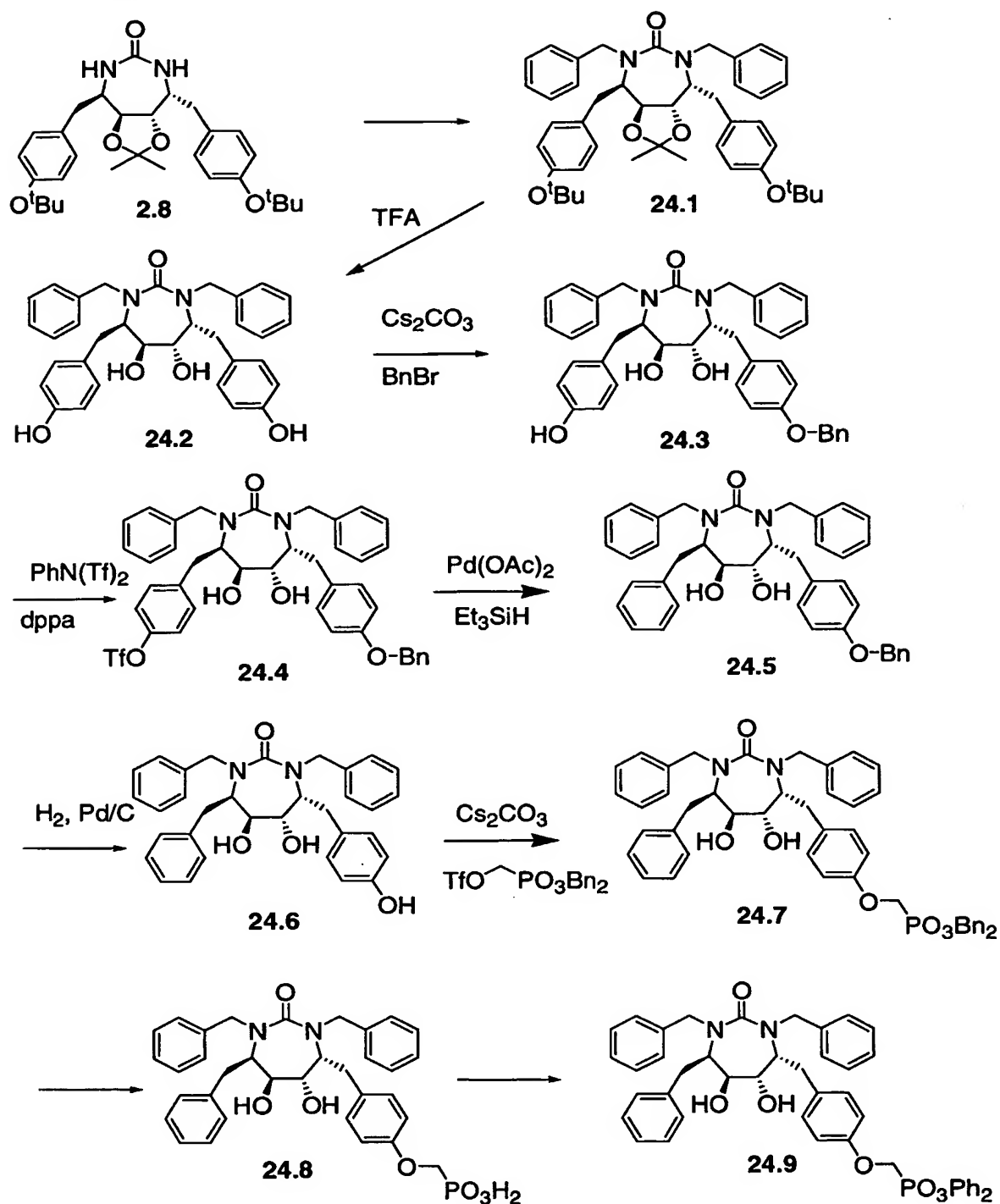
Dibenzyl phosphonate 23.7: To a solution of compound **23.6** (0.215 g, 0.563 mmol) in THF
30 (10 mL) was added dibenzyl triflate **3.11** (0.315 g, 0.74 mmol) and cesium carbonate (0.325g, 1 mmol). The mixture was stirred at room temperature for 2 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and

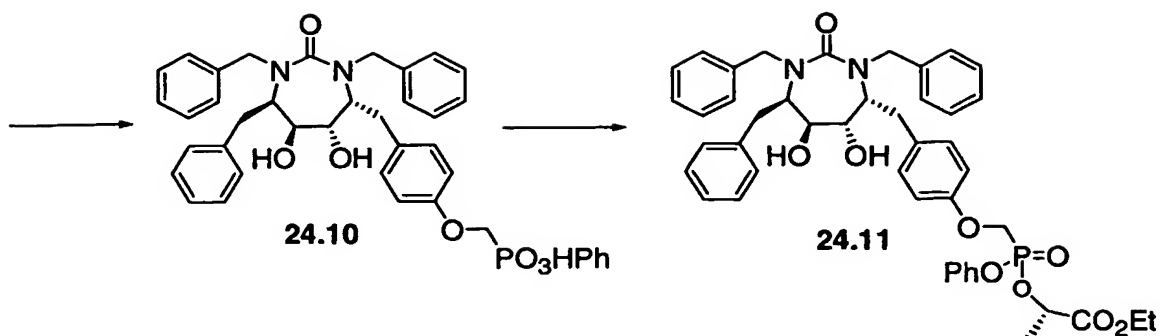
concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **23.7** (0.31 g, 84%).

Diphenyl ester 23.8: A solution of compound **23.7** (0.3 g, 0.457 mmol) and benzyl bromide (0.165 mL, 1.39 mmol) in THF (10 mL) was treated with potassium *tert*-butoxide (1M/THF, 1.2 mL) for 0.5 h. The mixture was diluted with ethyl acetate and washed with HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and treated with 10% Pd/C (0.05 g) under hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was treated with TFA (1 mL) in methanol (5 mL) for 1 h, and then concentrated under reduced pressure. The residue was dissolved in pyridine (1 mL) and mixed with phenol (0.45 g, 4.8 mmol) and 1,3-dicyclohexylcarbodiimide (0.38 g, 1.85 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound **23.8** (0.085 g, 24%).

Mono amidate 23.9: To a solution of **23.8** (0.085g, 0.11 mmol) in acetonitrile (1 mL) was added sodium hydroxide (1N, 0.25 mL) at 0°C. After stirred at 0°C for 1 h, the mixture was acidified with Dowex resin to pH = 3, and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (0.5 mL) and mixed with L-alanine ethyl ester hydrochloride (0.062 g, 0.4 mmol) and 1,3-dicyclohexyl-carbodiimide (0.125 g, 0.6 mmol). The mixture was stirred at 60°C for 0.5 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by HPLC (C-18, 65% acetonitrile / water) to afford compound **23.9** (0.02 g, 23%). ¹H NMR (CDCl₃): δ 1.2 (m, 3H), 1.4 (m, 3H), 1.8 (brs, 2H), 2.8-3.1 (m, 6H), 3.5-3.7 (m, 4H), 3.78 (m, 1H), 4.0-4.18 (m, 2H), 4.2-4.4 (m, 3H), 4.9 (m, 2H), 6.8-7.4 (m, 24H). ³¹P NMR (CDCl₃): δ 20.9, 19.8. MS: 792 (M+1).

Scheme 24





- Di-tert butyl ether 24.1:** To a solution of compound 2.8 (0.51 g, 1 mmol) and benzyl bromide (0.43g, 2.5 mmol) in THF (6 mL) was added potassium *tert*-butoxide (1M/THF, 2.5 mL). The mixture was stirred at room temperature for 0.5 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound 24.1 (0.62 g, 90%).
- Diol 24.2:** To a solution of compound 24.1 (0.62 g, 0.9 mmol) in methylene chloride (4 mL) was added TFA (1 mL) and water (0.1 mL). The mixture was stirred for 2 h, and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound 24.2 (0.443g, 92%).
- Benzyl ether 24.3:** Compound 24.3 was prepared in 46% yield according to the procedure described in Scheme 23 for the preparation of 23.3.
- Triflate 24.4:** Compound 24.4 was prepared in 95% yield according to the procedure described in Scheme 23 for the preparation of 23.4.
- Benzyl ether 24.5:** Compound 24.5 was prepared in 93% yield according to the procedure described in Scheme 23 for the preparation of 23.5.

Phenol 24.6: Compound **24.6** was prepared in 96% yield according to the procedure described in Scheme 23 for the preparation of **23.6** from **23.5**.

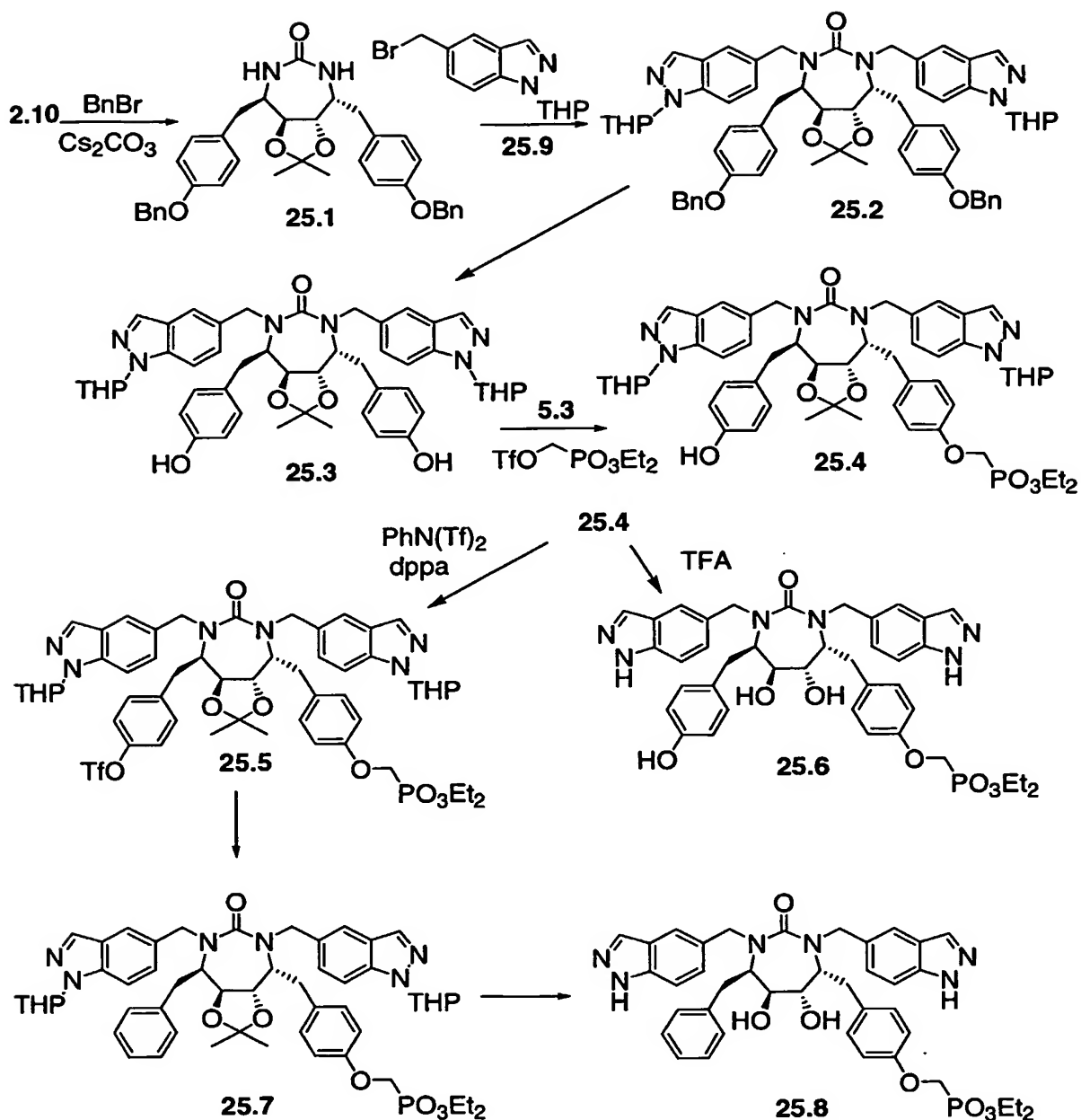
Dibenzyl phosphonate 24.7: Compound **24.7** was prepared in 82% yield according to the procedure described in Scheme 23 for the preparation of **23.7**.

Diacid 24.8: A solution of **24.7** (0.16 g, 0.207 mmol) in ethyl acetate (4 mL) and isopropyl alcohol (4 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 4 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **24.8** (0.125 g, 98%) as a white solid.

Diphenyl ester 24.9: To a solution of compound **24.8** (0.12 g, 0.195 mmol) in pyridine (1 mL) was added phenol (0.19 g, 2 mmol) and 1,3-dicyclohexylcarbodiimide (0.206 g, 1 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound **24.9** (0.038 g, 25%).

Mono lactate 24.11: Compound **24.9** was converted, via compound **24.10**, into compound **24.11** in 36% yield according to the procedure described in Scheme 23 for the preparation of **23.9** except utilizing the ethyl lactate ester in place of L-alanine ethyl ester. ¹H NMR (CDCl₃): δ 1.05 (t, J = 8 Hz, 1.5H), 1.1 (t, J = 8 Hz, 1.5H), 1.45 (d, J = 8 Hz, 1.5H), 1.55 (d, J = 8 Hz, 1.5H), 2.6 (brs, 2H), 2.9-3.1 (m, 6H), 3.5-3.65 (m, 4H), 4.15-4.25 (m, 2H), 4.4-4.62 (m, 2H), 4.9 (m, 2H), 5.2 (m, 1H), 6.9-7.4 (m, 24H). ³¹P NMR (CDCl₃): d 17.6, 15.5. MS: 793 (M+1).

Scheme 25



- 5 **Dibenzyl ether 25.1:** The protection reaction of compound 2.10 with benzyl bromide was carried out in the same manner as described in Scheme 23 to afford compound 25.1.

Bis indazole 25.2: The alkylation of compound 25.1 with bromide 25.9 was carried out in the same manner as described in Scheme 23 to afford compound 25.2 in 96% yield.

Diol 25.3: A solution of **25.2** (0.18 g, 0.178 mmol) in ethyl acetate (5 mL)) and isopropyl alcohol (5 mL) was treated with 20% Pd(OH)₂/C (0.09g) and stirred under a hydrogen atmosphere (balloon) for 24 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **25.3** in quantitative yield.

5

Diethyl phosphonate 25.4: To a solution of compound **25.3** (0.124 g, 0.15 mmol) in acetonitrile (8 mL) and DMF (1 mL) was added potassium tert-butoxide (0.15 mL, 1M/THF). The mixture was stirred for 10 min. to form a clear solution. Diethyl triflate **5.3** (0.045 g, 0.15 mmol) was added to the reaction mixture. After stirred for 0.5 h, the reaction mixture
10 was diluted with ethyl acetate and washed with HCl (0.1N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **25.4** (0.039 g, 55% (based on recovered starting material: 0.064 g, 52%).

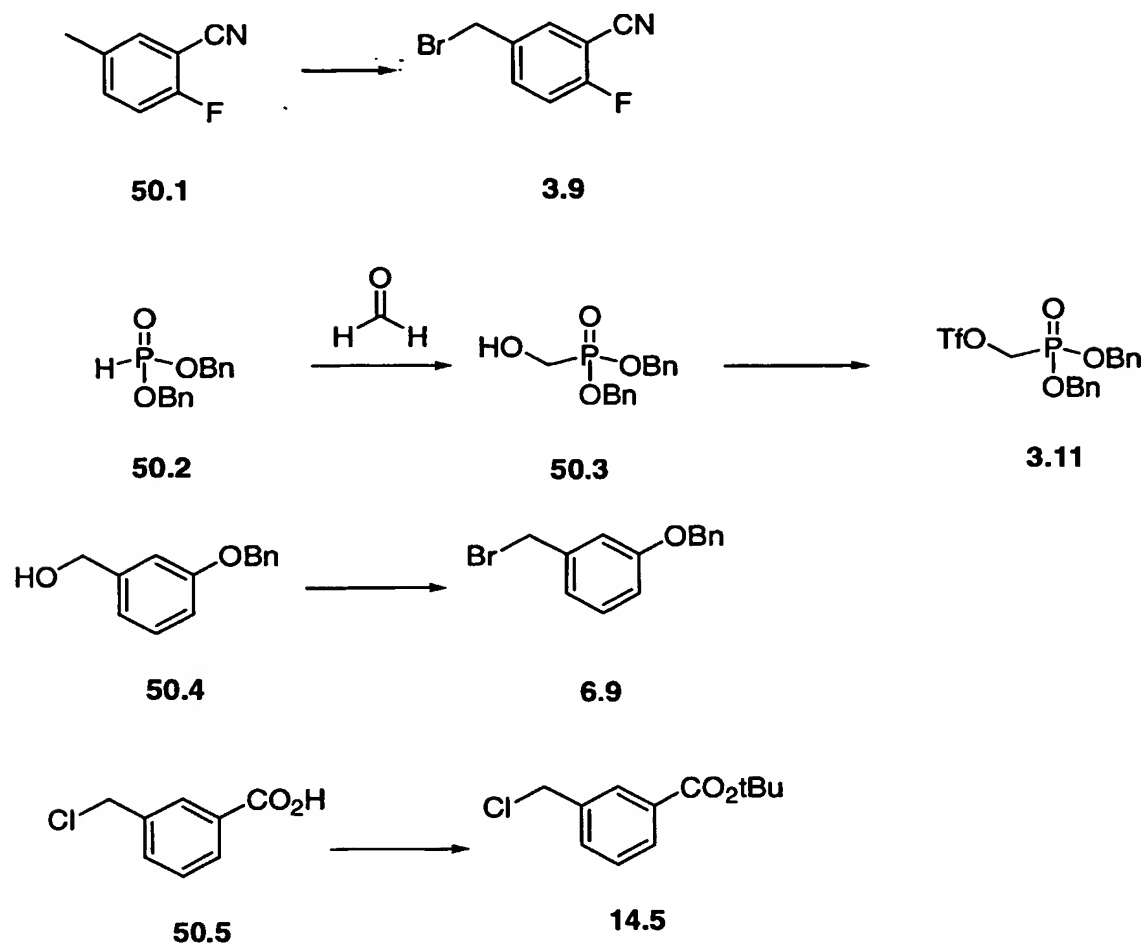
15 **Bisindazole 25.6:** A mixture of compound **25.4** (0.027 g), ethanol (1.5 mL), TFA (0.6 mL) and water (0.5 mL) was stirred at 60°C for 18 h. The mixture was concentrated under reduced pressure, and the residue was purified by HPLC to afford compound **25.6** as a TFA salt (0.014 g, 51%). ¹H NMR (CD₃OD): δ 1.4 (t, J = 8 Hz, 6H), 2.9 (M, 4H), 3.2 (m, 2H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.75 (d, J =
20 9 Hz, 2H), 6.9 (m, 4H), 7.0 (d, J = 9 Hz, 2H), 7.4-7.6 (m, 6H), 8.1 (brs, 2H). ³¹P NMR (CD₃OD): δ 20.8. MS: 769 (M+1).

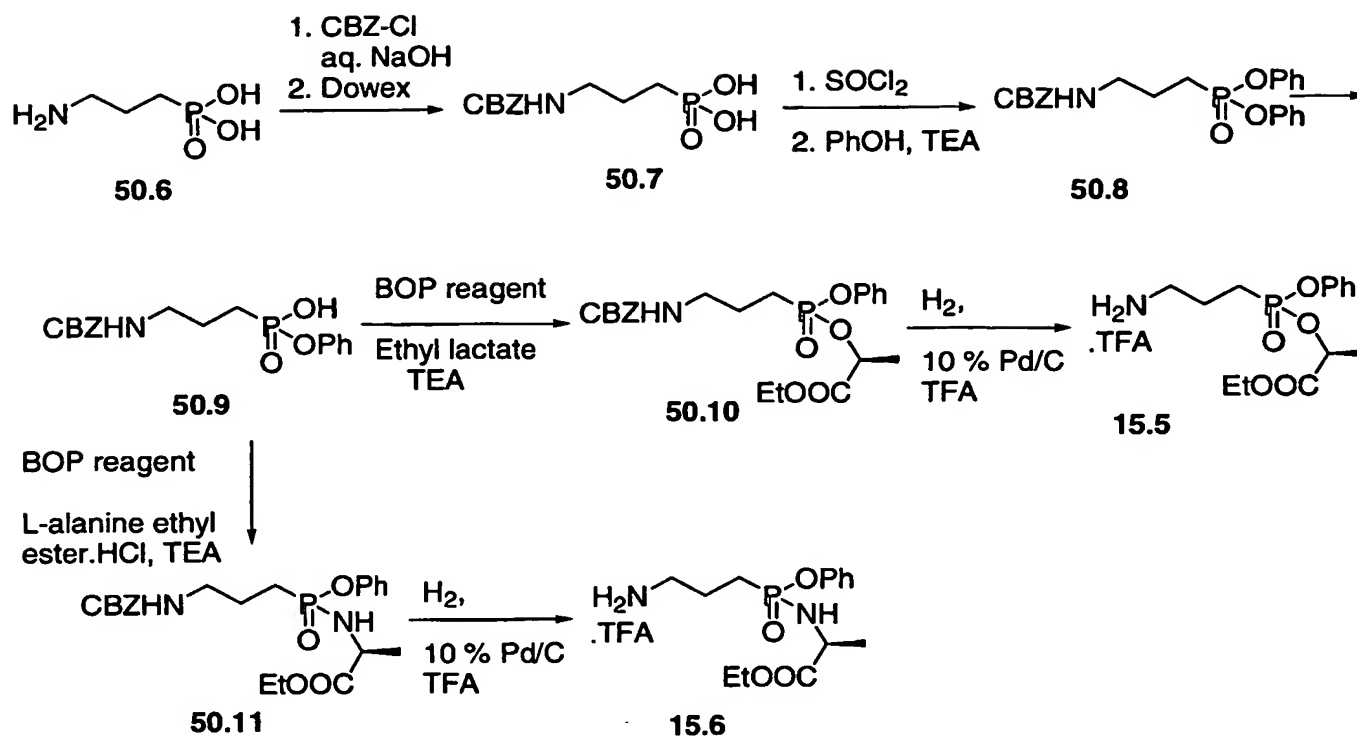
Diethyl phosphonate 25.7: Compound **25.4** was converted into compound **25.7** in 76% yield according to the procedures described in Scheme 23 for the conversion of **23.3** into
25 **23.5**.

Bis indazole 25.8: Compound **25.7** (0.029 g) was treated in the same manner as compound **25.4** in the preparation of **25.6** to afford compound **25.8** as a TFA salt (0.0175 g, 59%). ¹H NMR (CD₃OD): δ 1.4 (t, J = 8 Hz, 6H), 3.0 (M, 4H), 3.15 (d, J = 14 Hz, 1H), 3.25 (d, J = 14
30 Hz, 1H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.9 (d, J = 9 Hz, 2H), 7.0 (d, J = 9 Hz, 2H), 7.1 (d, J = 7 Hz, 2H), 7.2-7.6 (m, 9H), 8.1 (brs, 2H). ³¹P NMR (CD₃OD): δ 20.8. MS: 753 (M+1).

Preparation of Alkylating and Phosphonate Reagents

Scheme 50





3-cyano-4-fluoro-benzylbromide 3.9: The commercially available 2-fluoro-4-methylbenzonitrile **50.1** (10 g, 74 mmol) was dissolved in carbon tetrachloride (50 mL) and then treated with NBS (16 g, 90 mmol) followed by AIBN (0.6 g, 3.7 mmol). The mixture was stirred at 85°C for 30 min and then allowed to cool to room temperature. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by silica gel eluting with 5-20% ethyl acetate in hexanes to give **3.9** (8.8 g, 56%).

4-benzyloxy benzyl chloride 3.10 is purchased from Aldrich

Dibenzyl triflate 3.11: To a solution of dibenzyl phosphite **50.2** (100 g, 381 mmol) and formaldehyde (37% in water, 65 mL, 860 mmol) in THF (200 mL) was added TEA (5 mL, 36 mmol). The resulted mixture was stirred for 1 h, and then concentrated under reduced pressure. The residue was dissolved in methylene chloride and hexane (1:1, 300 mL), dried over sodium sulfate, filtered through a pad of silica gel (600 g) and eluted with ethyl acetate and hexane (1:1). The filtrate was concentrated under reduced pressure. The residue **50.3** (95 g) was dissolved in methylene chloride (800 mL), cooled to -78°C and then charged with pyridine (53 mL, 650 mmol). To this cooled solution was slowly added trifluoromethanesulfonic anhydride (120 g, 423 mmol). The resulted reaction mixture was

stirred and gradually warmed up to -15°C over 1.5 h period of time. The reaction mixture was cooled down to about -50°C , diluted with hexane-ethyl acetate (2:1, 500 mL) and quenched with aqueous phosphoric acid (1M, 100 mL) at -10°C to 0°C . The mixture diluted with hexane-ethyl acetate (2:1, 1000 mL). The organic phase was washed with water, dried
5 over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford dibenzyl triflate **3.11** (66 g, 41%) as a colorless oil.

Diethyl triflate 5.3 is prepared as described in Tet Lett. 1986, 27, p1477-1480

10 **3-Benzyloxybenzylbromide 6.9**: To a solution of triphenyl phosphine (15.7 g, 60 mmol) in THF (150 mL) was added a solution of carbon tetrabromide (20 g, 60 mmol) in THF (50 mL). A precipitation was formed and stirred for 10 min. A solution of 3-benzyloxybenzyl alcohol **50.4** (10 g, 46.7 mmol) was added. After stirred for 1.5 h, the reaction mixture was
15 filtered and concentrated under reduced pressure. The majority of triphenyl phosphine oxide was removed by precipitation from ethyl acetate-hexane. The crude product was purified by chromatography on silica gel and precipitation from hexane to give the desired product 3-Benzyloxybenzylbromide **6.9** (10 g, 77%) as a white solid.

20 **t-Butyl-3-chloromethyl benzoate 14.5**: A benzene solution (15 ml) of 3-chloromethylbenzoic acid **50.5** (1 g, 5.8 mmol) was heated at reflux, followed by the slow addition of N,N-dimethylformamide-di-t-butylacetal (5 m). The resulting solution was refluxed for 4 h, concentrated under reduced pressure and purified by silica gel column to afford **14.5** (0.8 g, 60 %).

25 **Aminopropyl-diethylphosphonate 14.6** is purchased from Acros

Aminoethyl-diethylphosphonate oxalate 14.7 is purchased from Acros

30 **Aminopropyl-phenol-ethyl lactate phosphonate 15.5**

N-CBZ-aminopropyl diphenylphosphonate 50.8: An aqueous sodium hydroxide solution (50 mL of 1 N solution, 50 mmol) of 3-aminopropyl phosphonic acid **50.6** (3 g, 1.5 mmol)

was reacted with CBZ-Cl (4.1 g, 24 mmol) at room temperature overnight. The reaction mixture was washed with methylene chloride, acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to dryness. The crude N-CBZ-aminopropyl phosphonic acid **50.7** (5.8 mmol) was suspended in CH₃CN (40 mL), and reacted with thionyl chloride (5.2 g, 44 mmol) at reflux for 4 hr, concentrated, and azeotroped with CH₃CN twice. The reaction mixture was redissolved in methylene chloride (20 mL), followed by the addition of phenol (3.2 g, 23 mmol), was cooled to 0°C. To this 0°C cold solution was added TEA (2.3 g, 23 mmol), and stirred at room temperature overnight. The reaction mixture was concentrated and purified on silica gel column chromatograph to afford **50.8** (1.5 g, 62 %).

Monophenol derivative 50.9: A CH₃CN solution (5 mL) of **50.8** (0.8 g, 1.88 mmol) was cooled to 0°C, and treated with 1N NaOH aqueous solution (4 mL, 4 mmol) for 2 h. The reaction was diluted with water, extracted with ethyl acetate, acidified with Dowex 50wx8-200. The aqueous solution was concentrated to dryness to afford **50.9** (0.56 g, 86%).

Monolactate derivative 50.10: A DMF solution (1 mL) of crude **50.9** (0.17 g, 0.48 mmol), BOP reagent (0.43 g, 0.97 mmol), ethyl lactate (0.12 g, 1 mmol), and DIPEA (0.31 g, 2.4 mmol) was reacted for 4 hr at room temperature. The reaction mixture was partitioned between methylene chloride and 5 % citric acid aqueous solution. The organic solution was separated, concentrated, and purified on preparative TLC to give **50.10** (0.14 g, 66%).

3-Aminopropyl lactate phosphonate 15.5: An ethyl acetate/ethanol solution (10 mL/2 mL) of **50.10** (0.14 g, 0.31 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (40 mg) for 3 hr. The catalyst was filtered off. The filtrate was concentrated to dryness to afford **15.5** (0.14 g, quantitative). NMR (CDCl₃): δ 8.0-8.2 (b, 3H), 7.1-7.4 (m, 5H), 4.9-5.0 (m, 1H), 4.15-4.3 (m, 2H), 3.1-3.35 (m, 2H), 2.1-2.4 (m, 4H), 1.4 (d, 3H), 1.3 (t, 3H).

Aminopropyl-phenol-ethyl alanine phosphonate 15.6: Compound **15.6** (80 mg) was prepared from the reaction of **50.9** (160 mg, 0.45 mmol) and L-alanine ethyl ester hydrochloride salt (0.11g, 0.68 mmol) in the presence of DIPEA and BOP reagent to give **50.11**, followed by the hydrogenation in the presence of 10% Pd/C and TFA to yield **15.6**. NMR (CDCl₃ + ~10 % CD₃OD): δ 8.0-8.2 (b), 7.25-7.35 (t, 2H), 7.1-7.2 (m, 3H), 4.0-4.15

(m, 2H), 3.8-4.0 (m, 1H), 3.0-3.1 (m, 2H), 1.15-1.25 (m, 6H). P NMR (CDCl_3 + ~10 % CD_3OD): 32.1 & 32.4 ppm.

Aminopropyl dibenzyl phosphonate 15.7 :

5

N-BOC-3-aminopropyl phosphonic acid 50.13: A THF-1N aqueous solution (16 mL-16 mL) of 3-aminopropyl phosphonic acid **50.12** (1 g, 7.2 mmol) was reacted with $(\text{BOC})_2\text{O}$ (1.7 g, 7.9 mmol) overnight at room temperature. The reaction mixture was concentrated, and partitioned between methylene chloride and water. The aqueous solution was acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to give **50.13** (2.2 g, 92 %).

10

N-BOC-3-aminopropyl dibenzyl phosphonate 50.14: A CH_3CN solution (10 mL) of **50.13** (0.15 g, 0.63 mmol), cesium carbonate (0.61 g, 1.88 mmol), and benzyl bromide (0.24 g, 1.57 mmol) was heated at reflux overnight. The reaction mixture was cooled to room temperature, and diluted with methylene chloride. The white solid was filtered off, washed thoroughly with methylene chloride. The organic phase was concentrated, and purified on preparative TLC to give **50.14** (0.18 g, 70%). MS: 442 (M + Na).

15

Aminopropyl dibenzyl phosphonate 15.7: A methylene chloride solution (1.6 mL) of **50.14** (0.18 g) was treated with TFA (0.4 mL) for 1 hr. The reaction mixture was concentrated to dryness, and azeotroped with CH_3CN twice to afford **15.7** (0.2 g, as TFA salt). NMR (CDCl_3): δ 8.6 (b, 2H), 7.9 (b, 2H), 7.2-7.4 (m, 10H), 4.71-5.0 (2 abq, 4H), 3.0 (b, 2H), 1.8-2 (m, 4H). ^{31}P NMR (CDCl_3): 32.0 ppm. F NMR (CDCl_3): -76.5 ppm.

20

25

Aminomethyl diethylphosphonate 22.8 is purchased from Acros

Bromomethyl, tetrahydropyran indazole 25.9 is prepared according to J. Org. Chem. 1997, 62, p5627

30

Activity of the CCPPI Compounds

The enzyme inhibitory potency (K_i), antiviral activity (EC_{50}), and cytotoxicity (CC_{50}) of the tested compounds were measured and demonstrated.

Biological assays used for the characterization of PI prodrugs**HIV-1 Protease Enzyme Assay (K_i)**

The assay is based on the fluorimetric detection of synthetic hexapeptide substrate cleavage by HIV-1 protease in a defined reaction buffer as initially described by M.V.Toth and G.R.Marshall, Int. J. Peptide Protein Res. 36, 544 (1990)

Substrate: (2-aminobenzoyl)Thr-Ile-Nle-(p-nitro)Phe-Gln-Arg

Substrate supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-2992)

Enzyme: recombinant HIV-1 protease expressed in E.Coli

Enzyme supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-9040)

Reaction buffer: 100 mM ammonium acetate, pH 5.3

1 M sodium chloride

1 mM ethylenediaminetetraacetic acid

1 mM dithiothreitol

10% dimethylsulfoxide

Assay protocol for the determination of inhibition constant K_i :

1. Prepare series of solutions containing identical amount of the enzyme (1 to 2.5 nM) and a tested inhibitor at different concentrations in the reaction buffer
2. Transfer the solutions (190 μ L each) into a white 96-well plate
3. Preincubate for 15 min at 37°C
4. Solubilize the substrate in 100% dimethylsulfoxide at a concentration of 800 μ M. Start the reaction by adding 10 μ L of 800 μ M substrate into each well (final substrate concentration of 40 μ M)
5. Measure the real-time reaction kinetics at 37°C by using Gemini 96-well plate fluorimeter (Molecular Devices, Sunnyvale, CA) at $\lambda(\text{Ex}) = 330$ nm and $\lambda(\text{Em}) = 420$ nm
6. Determine initial velocities of the reactions with different inhibitor concentrations and calculate K_i (in picomolar concentration units) value by using EnzFitter program

(Biosoft, Cambridge, U.K.) according to an algorithm for tight-binding competitive inhibition described by Ermoloeff J., Lin X., and Tang J., Biochemistry 36, 12364 (1997)

Anti-HIV-1 Cell Culture Assay (EC_{50})

- 5 The assay is based on quantification of the HIV-1-associated cytopathic effect by a colorimetric detection of the viability of virus-infected cells in the presence or absence of tested inhibitors. The HIV-1-induced cell death is determined using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) which is converted only by intact cells into a product with specific absorption characteristics as
- 10 described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).

Assay protocol for determination of EC_{50} :

1. Maintain MT2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum
15 and antibiotics.
2. Infect the cells with the wild-type HIV-1 strain IIIB (Advanced Biotechnologies, Columbia, MD) for 3 hours at 37°C using the virus inoculum corresponding to a multiplicity of infection equal to 0.01.
3. Prepare a set of solutions containing various concentrations of the tested inhibitor by
20 making 5-fold serial dilutions in 96-well plate (100 μ L/well). Distribute the infected cells into the 96-well plate (20,000 cells in 100 μ L/well). Include samples with untreated infected and untreated mock-infected control cells.
4. Incubate the cells for 5 days at 37°C.
5. Prepare XTT solution (6 mL per assay plate) at a concentration of 2mg/mL in a
25 phosphate-buffered saline pH 7.4. Heat the solution in water-bath for 5 min at 55°C. Add 50 μ L of N-methylphenazonium methasulfate (5 μ g/mL) per 6 mL of XTT solution.
6. Remove 100 μ L media from each well on the assay plate.
7. Add 100 μ L of the XTT substrate solution per well and incubate at 37°C for 45 to 60 min in a CO₂ incubator.
- 30 8. Add 20 μ L of 2% Triton X-100 per well to inactivate the virus.
9. Read the absorbance at 450 nm with subtracting off the background absorbance at 650 nm.

10. Plot the percentage absorbance relative to untreated control and estimate the EC_{50} value as drug concentration resulting in a 50% protection of the infected cells.

Cytotoxicity Cell Culture Assay (CC_{50}):

- 5 The assay is based on the evaluation of cytotoxic effect of tested compounds using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).
- 10 *Assay protocol for determination of CC_{50} :*
1. Maintain MT-2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
 2. Prepare a set of solutions containing various concentrations of the tested inhibitor by making 5-fold serial dilutions in 96-well plate (100 μ L /well). Distribute cells into the
 - 15 96-well plate (20,000 cells in 100 μ L/well). Include samples with untreated cells as a control.
 3. Incubate the cells for 5 days at 37°C.
 4. Prepare XTT solution (6 mL per assay plate) in dark at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in a water-bath at 55°C for 5 min.
 - 20 Add 50 μ L of N-methylphenazonium methasulfate (5 μ g/mL) per 6 mL of XTT solution.
 5. Remove 100 μ L media from each well on the assay plate and add 100 μ L of the XTT substrate solution per well. Incubate at 37°C for 45 to 60 min in a CO₂ incubator.
 6. Add 20 μ L of 2% Triton X-100 per well to stop the metabolic conversion of XTT.
 7. Read the absorbance at 450 nm with subtracting off the background at 650 nm.
 - 25 8. Plot the percentage absorbance relative to untreated control and estimate the CC_{50} value as drug concentration resulting in a 50% inhibition of the cell growth. Consider the absorbance being directly proportional to the cell growth.

30 Resistance Evaluation (I50V and I84V/L90M fold change)

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a mutant HIV-1 strain

containing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus (EC_{50}) to a particular tested compound is measured by using the XTT-based cytopathic assay as described above. The degree of resistance to a tested compound is calculated as fold difference in EC_{50} between the wild type and a specific mutant virus. This represents a standard approach for HIV drug resistance evaluation as documented in various publications (e.g. Maguire et al., *Antimicrob. Agents Chemother.* 46: 731, 2002; Gong et al., *Antimicrob. Agents Chemother.* 44: 2319, 2000; Vandamme and De Clercq, in *Antiviral Therapy* (Ed. E. De Clercq), pp. 243, ASM Press, Washington, DC, 2001).

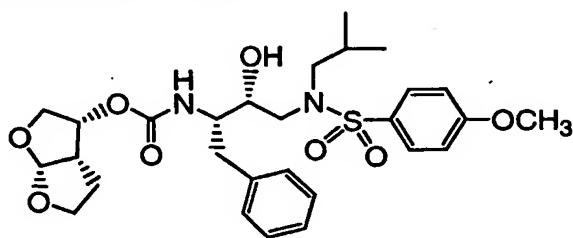
HIV-1 strains used for the resistance evaluation:

Two strains of mutant viruses containing I50V mutation in the protease gene have been used in the resistance assays: one with M46I/I47V/I50V mutations (designated I50V #1) and the other with L10I/M46I/I50V (designated I50V #2) mutations in the viral protease gene. A third virus with I84V/L90M mutations was also employed in the resistance assays. Mutants I50V #1 and I84V/L90M were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment of mutated viral protease gene that has been generated by PCR amplification. An approach similar to that described by Shi and Mellors in *Antimicrob. Agents Chemother.* 41: 2781-85, 1997 was used for the construction of mutant viruses from the generated DNA fragments. Mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After verification of protease gene sequence and determination of the infectious virus titer, the viral stock was used for drug resistance studies. Mutant I50V #2 is an amprenavir-resistant HIV-1 strain selected *in vitro* from the wild-type IIIB strain in the presence of increasing concentration of amprenavir over a period of > 9 months using an approach similar to that described by Partaledis et al., *J. Virol.* 69: 5228-5235, 1995. Virus capable of growing in the presence of 5

μM amprenavir was harvested from the supernatant of infected cells and used for resistance assays following the titration and protease gene sequencing.

Example 37: Activity of the Tested Compounds

- 5 The enzyme inhibitory potency (K_i), antiviral activity (EC_{50}), and cytotoxicity (CC_{50}) of the tested compounds are summarized in Table 1.



94-003

Table 1: Enzyme inhibition activity (Ki), antiviral cell culture activity (EC50), and cytotoxicity (CC50) of the tested compounds.

| Substitution of (P1)phenyl | Compound | Phosphonate substitution | HIV-1 protease inhibition Ki [pM] | Anti-HIV-1 Cell Culture Activity EC50 [nM] | Cytotoxicity CC50 [μ M] |
|----------------------------|------------|--------------------------|-----------------------------------|--|------------------------------|
| none | Amprenavir | none | 45.6 \pm 18.2 | 16 \pm 2.2 | |
| none | 94-003 | none | 1.46 \pm 0.58 | 1.4 \pm 0.3 | |
| phosphonyl | 27 | diacid | 11.8 \pm 6.0 | > 100,000 | > 100 |
| | 28 | diethyl | 1.2 \pm 0.8 | 5.0 \pm 2.8 | 70 |
| phosphonyl methoxy | 11 | diacid | 2.1 \pm 0.2 | 4,800 \pm 1,800 | > 100 |
| | 13 | diethyl | 2.6 \pm 1.5 | 3.0 \pm 0 | 50 |
| | 14 | dibenzyl | 12.7 \pm 1.9 | 2.3 \pm 0.4 | 35 |
| | 16c | bis(Ala-ethylester) | 15.4 \pm 0.85 | 105 \pm 43 | 60 |
| | 16d | bis(Ala-butylester) | 18.75 \pm 3.04 | 6.0 \pm 1.4 | |
| | 16e | bis(ABA-ethylester) | 8.8 \pm 1.7 | 12.5 \pm 3.5 | |
| | 16f | bis(ABA-butylester) | 3.5 \pm 1.4 | 4.8 \pm 1.8 | |
| | 16a | bis(Gly-ethylester) | 29 \pm 8.2 | 330 \pm 230 | |
| | 16b | bis(Gly-butylester) | 4.9 \pm 1.8 | 17.5 \pm 10.5 | |
| | 16g | bis(Leu-ethylester) | 29 \pm 9 | 6.8 \pm 0.4 | |
| | 16h | bis(Leu-butylester) | 31.7 \pm 19.3 | 120 \pm 42 | |
| | 16i | bis(Phe-ethylester) | | 17 \pm 12 | |
| | 16j | bis(Phe-butylester) | | 35 \pm 7 | |
| | 15 | bis(POC) | 36 | 825 \pm 106 | |
| | 11 | Monoethyl, monoacid | 0.45 \pm 0.15 | 700 \pm 0 | |

5 Cross-Resistance Profile Assay

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a recombinant HIV-1 strain expressing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus to a particular tested compound is measured by using the XTT-based cytopathic assay as described in Example B. The degree of resistance to a tested compound is calculated as fold difference in EC50 between the wild type and a specific mutant virus.

Recombinant HIV-1 strains with resistance mutations in the protease gene:

One mutant virus (82T/84V) was obtained from NIH AIDS Research and Reference Reagent Program (Rockville, MD). Majority of the mutant HIV-1 strains were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations selected during antiretroviral therapy with various protease inhibitors.

Additional mutant HIV-1 strains were constructed by a modified procedure relying on a homologous recombination of only two overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with only the protease gene deleted, and 2. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations. In both cases, mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After determination of the virus titer the virus stock was used for drug resistance studies.

Example 39: Cross-Resistance Profile of the Tested Compounds

Cross-resistance profile of currently used HIV-1 protease inhibitors was compared with that of the newly invented compounds (Table 2).

Table 2. Cross-resistance profile of HIV-1 protease inhibitors

| Compound | EC 50 [nM] | Fold Change in EC ₅₀ Relative to WT HIV-1 | | | | | | | | | | | Total No. of Resistant Viruses ^b |
|------------|------------|--|------------|--------------------------|-------------------|--------------------------|--------------------------|-------------------|---------------------------------|---------------------------------|--------------------------|---------------------------------|---|
| | | 8K ^a 46I 90M | 46I 84A | 10I 48V 54V 82A | 46I 47V 50V | 10R 46I 82T 84V | 30N 50S 82I 88D | 54V 71V 82S | 10F 46I 71V 82T 90M | 10I 48V 71V 82A 90M | 48V 54V 71V 82S | 10I 84V 71V 73S 90M | |
| Amprenavir | 20 | 1.25 | 14 | 2 | 38 | 4 | 0.8 | 4 | 13 | 2.5 | 2 | 10 | 4 |
| Nelfinavir | 14 | 13 | 11 | 11.5 | 2 | 3 | 43 | 12 | 33 | 27 | 12 | 65 | 9 |
| Indinavir | 15 | 4 | 10 | 15 | nd | 7 | 1 | 10 | 13 | 28 | 23 | 43 | 8 |
| Ritonavir | 15 | 34 | 18 | 20 | 13 | 47 | 2 | 20 | 32 | 22 | >50 | 42 | 10 |
| Saquinavir | 4 | 1 | 2.5 | 11 | 1 | 2.5 | 1 | 3 | 2.5 | 12 | 45 | 40 | 4 |
| Lopinavir | 8 | nd | 9 | nd | 19 | 11 | nd | nd | 7.5 | 4.5 | 60 | 11 | 6 |
| Tipranavir | 80 | nd | 1 | 0.4 | 0.5 | 5 | 0.5 | 3.5 | 3 | 0.3 | 2 | nd | 1 |
| 94-003 | 0.5 | nd | 8 | 0.5 | 29 | nd | 0.4 | 3.5 | nd | nd | nd | 8 | 3 |
| | | | | | | | | | | | | | |
| GS 16503 | 16 | 1.2 | 1 | 0.4 | 3.3 | 1 | 0.6 | 0.9 | 1 | 0.4 | 0.5 | 2 | 0 |
| GS 16571 | 22 | 1.8 | 1 | 0.3 | 0.8 | 0.6 | 0.7 | 0.6 | 0.8 | 0.2 | 0.2 | 0.9 | 0 |
| GS 16587 | 15 | 1.5 | 1 | 0.5 | 2 | 1 | 1 | 0.9 | 1 | 0.4 | 0.4 | 1 | 0 |

5 ^a Resistance-associated mutations present in the viral protease. The highlighted changes represent primary resistance mutations.

^b Resistance is considered as a 5-fold and higher change in the EC₅₀ value of the mutant virus relative to the wild-type virus.

Example Section N**Plasma and PBMC Exposure Following Intravenous and Oral Administration of Prodrug to Beagle Dogs**

The pharmacokinetics of a phosphonate prodrug GS77366 (P1-monoLac-iPr), its active metabolite (metabolite X, or GS77568), and GS8373 were studied in dogs following intravenous and oral administration of the prodrug.

Dose Administration and Sample Collection. The in-life phase of this study was conducted in accordance with the USDA Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and followed the standards for animal husbandry and care found in the Guide for the Care and Use of Laboratory Animals, 7th Edition, Revised 1996. All animal housing and study procedures involving live animals were carried out at a facility which had been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care - International (AAALAC).

Each animal in a group of 4 female beagle dogs was given a bolus dose of GS77366 (P1-monoLac-iPr) intravenously at 1 mg/kg in a formulation containing 40% PEG 300, 20% propylene glycol and 40% of 5% dextrose. Another group of 4 female beagle dogs was dosed with GS77366 via oral gavage at 20 mg/kg in a formulation containing 60% Vitamin-E TPGS, 30% PEG 400 and 10% propylene glycol.

Blood samples were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-dose. Plasma (0.5 to 1 mL) was prepared from each sample and kept at -70°C until analysis. Blood samples (8 mL) were also collected from each dog at 2, 8 and 24 hr post dose in Becton-Dickinson CPT vacutainer tubes. PBMCs were isolated from the blood by centrifugation for 15 minutes at 1500 to 1800 G. After centrifugation, the fraction containing PBMCs was transferred to a 15 mL conical centrifuge tube and the PBMCs were washed twice with phosphate buffered saline (PBS) without Ca²⁺ and Mg²⁺. The final wash of the cell pellet was kept at -70°C until analysis.

Measurement of the prodrug, metabolite X and GS8373 in plasma and PBMCs. For plasma sample analysis, the samples were processed by a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10 µM, from

J.T. Baker) were conditioned with 200 μ L of methanol followed by 200 μ L of water. An aliquot of 200 μ L of plasma sample was applied to each cartridge, followed by two washing steps each with 200 μ L of deionized water. The compounds were eluted from the cartridges with a two-step process each with 125 μ L of methanol. Each well was added 50 μ L of water and mixed. An aliquot of 25 μ L of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5 μ m) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, pH 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, pH 4.6. The chromatography was carried out at a flow rate of 250 μ L/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure GS77366, GS8373 and Metabolite X with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for GS77366, GS8373 and GS77568 (Metabolite X) in plasma.

For PBMC sample analysis, phosphate buffered saline (PBS) was added to each PBMC pellet to bring the total sample volume to 500 μ L in each sample. An aliquot of 150 μ L from each PBMC sample was mixed with an equal volume of methanol, followed by the addition of 700 μ L of 1% formic acid in water. The resulting mixture was applied to a Speedisk C18 solid phase extraction cartridge (1 mL, 20 mg, 10 μ m, from J.T. Baker) which had been conditioned as described above. The compounds were eluted with methanol after washing the cartridge 3 times with 10% methanol. The solvent was evaporated under a stream of N₂, and the sample was reconstituted in 150 μ L of 30% methanol. An aliquot of 75 μ L of the solution was injected for LC/MS/MS analysis. The limit of quantitation was 0.1 ng/mL in the PBMC suspension.

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were calculated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

Plasma and PBMC Concentration-time Profiles.

The concentration-time profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous dosing of GS77366 were compared at 1 mg/kg in dogs. The data demonstrate that the prodrug can effectively deliver the active components (metabolite X and GS8373) into cells that are primarily responsible for HIV replication, and that the active components in these cells had much longer half-life than in plasma.

The pharmacokinetic properties of GS77568 in PBMCs following oral administration of GS77366 in dogs are compared with that of nelfinavir and amprenavir, two marketed HIV protease inhibitors (Table 3). These data show that the active component (GS77568) from the phosphonate prodrug had sustained levels in PBMCs compared to nelfinavir and amprenavir.

Table 3. Comparison of GS77568 with nelfinavir and amprenavir in PBMCs following oral administration in beagle dogs.

| Compound | Dose | $t_{1/2}$ (hr) | AUC _(2-24 hr) |
|------------|---------------------|----------------|--------------------------|
| Nelfinavir | 17.5 mg/kg | 3.0 hr | 33,000 nM·hr |
| Amprenavir | 20 mg/kg | 1.7 hr | 102,000 nM·hr |
| GS77568 | 20 mg/kg of GS77366 | > 20 hr | 42,200 nM·hr |

Example Section O**Intracellular Metabolism/In Vitro Stability****5 1. Uptake and Persistence in MT2 cells, quiescent and stimulated PBMC**

The protease inhibitor (PI) phosphonate prodrugs undergo rapid cell uptake and metabolism to produce acid metabolites including the parent phosphonic acid. Due to the presence of charges, the acid metabolites are significantly more persistent in the cells than non-charged PI's. In order to estimate the relative intracellular levels of the different PI prodrugs, three
10 compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate, monoamidate phenoxy phosphonate and monolactate phenoxy phosphonate (Figure 1) were incubated at 10 μ M for 1 hr with MT-2 cells, stimulated and quiescent peripheral blood mononuclear cells (PBMC) (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 24 hr (chase phase). At
15 specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. Typically, the cell lysates were centrifuged and 100 μ L of the supernatant were mixed with 200 μ L of 7.5 μ M amprenavir (Internal Standard) in 80% acetonitrile/20% water and injected into an HPLC system (70 μ L).

20 HPLC Conditions:

Analytical Column: Prodigy ODS-3, 75 x 4.6, 3 μ + C18 guard at 40°C

Gradient:

Mobile Phase A: 20 mM ammonium acetate in 10% ACN/90% H₂O

Mobile Phase B: 20 mM ammonium acetate in 70% ACN/30% H₂O

25 30-100%B in 4 min, 100%B for 2 min, 30%B for 2 min at 2.5 mL/min.

Run Time: 8 min

UV Detection at 245 nm

Concentrations of Intracellular metabolites were calculated based on cell volume 0.2 μ L/mLn
30 cells for PBMC and 0.338 μ L / mLn (0.676 μ L / mL) for MT-2 cells.

Chemical Structures of Selected Protease Inhibitor Phosphonate Prodrugs and Intracellular Metabolites:

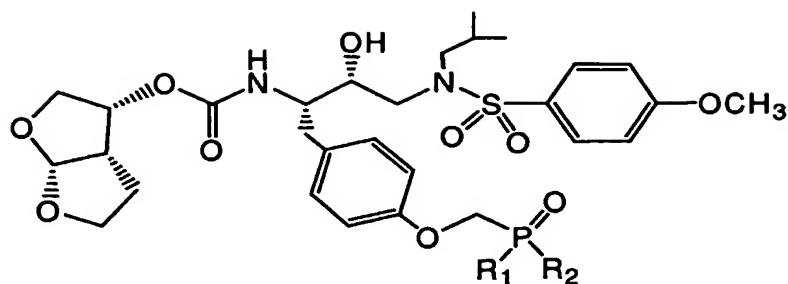


Table 4:

| GS No. | R1 | R2 | EC ₅₀ (nM) |
|--------|-----------------------------|---|-----------------------|
| 8373 | OH | OH | 4,800±1,800 |
| 16503 | HNCH(CH ₃)COOBu | HNCH(CH ₃)COOBu | 6.0±1.4 |
| 16571 | OPh | HNCH(CH ₃)COOEt | 15±5 |
| 17394 | OPh | OCH(CH ₃)COOEt | 20±7 |
| 16576 | OPh | HNCH(CH ₂ CH ₃)COOEt | 12.6±4.8 |
| Met X | OH | HNCH(CH ₃)COOH | >10,000 |
| Met LX | OH | OCH(CH ₃)COOEt | 1750±354 |

- 5 A significant uptake and conversion of all 3 compounds in all cell types was observed (Table 4). The uptake in the quiescent PBMC was 2-3-fold greater than in the stimulated cells. GS-16503 and GS-16571 were metabolized to Metabolite X and GS-8373. GS-17394 metabolized to the Metabolite LX. Apparent intracellular half-lives were similar for all metabolites in all cell types (7-12 hr). A persistence of Total Acid Metabolites of Protease Inhibitor Prodrugs in Stimulated (A), Quiescent PBMC (B) and MT-2 Cells (C) (1 hr, 10 μ M Pulse, 24 hr Chase) was observed.

2. Uptake and Persistence in Stimulated and Quiescent T-cells

- 15 Since HIV mainly targets T-lymphocytes, it is important to establish the uptake, metabolism and persistence of the metabolites in the human T-cells. In order to estimate the relative intracellular levels of the different PI prodrugs, GS-16503, 16571 and 17394 were incubated at 10 μ M for 1 hr with quiescent and stimulated T-cells (pulse phase). The prodrugs were compared with a non-prodrug PI, nelfinavir. After incubation, the cells were washed, resuspended in the cell culture media and incubated for 4 hr (chase phase). At specific time

points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC.

- 5 Table 5 demonstrate the levels of total acid metabolites and corresponding prodrugs in T-cells following pulse/chase and continuous incubation. There was significant cell uptake/metabolism in T-lymphocytes. There was no apparent difference in uptake between stimulated and quiescent T-lymphocytes. There was significantly higher uptake of phosphonate PI's than nelfinavir. GS17394 demonstrates higher intracellular levels than
- 10 GS16571 and GS16503. The degree of conversion to acid metabolites varied between different prodrugs. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equal mixture of the mono-phosphonic acid metabolite and GS-8373 except for GS-17394, where Metabolite LX was stable, with no GS-8373 formed.

15

Table 5. Intracellular Levels of Metabolites and Intact Prodrug Following Continuous and 1 hr Pulse/4 hr Chase Incubation (10 μ M/0.7 mLn cells/1 mL) of 10 μ M PI Prodrugs and Nelfinavir with Quiescent and Stimulated T-cell

| Compound | Time (h) | Continuous Incubation | | | | 1 hr Pulse /4 hr Chase | | | |
|------------|----------|-----------------------|--------------------|---------------------|--------------------|------------------------|--------------------|---------------------|--------------------|
| | | Quiescent T-cells | | Stimulated T-cells | | Quiescent T-cells | | Stimulated T-cells | |
| | | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) |
| 16503 | 0 | 1180 | 42 | 2278 | 0 | 2989 | 40 | 1323 | 139 |
| | 2 | 3170 | 88 | 1083 | 116 | 1867 | 4 | 1137 | 31 |
| | 4 | 5262 | 0 | 3198 | 31 | 1054 | 119 | 1008 | 0 |
| 16571 | 0 | 388 | 1392 | 187 | 1417 | 1042 | 181 | 858 | 218 |
| | 2 | 947 | 841 | 1895 | 807 | 1170 | 82 | 1006 | 35 |
| | 4 | 3518 | 464 | 6147 | 474 | 1176 | 37 | 616 | 25 |
| 17394 | 0 | 948 | 1155 | 186 | 1194 | 4480 | 14 | 2818 | 10 |
| | 2 | 7231 | 413 | 3748 | 471 | 2898 | 33 | 1083 | 51 |
| | 4 | 10153 | 167 | 3867 | 228 | 1548 | 39 | 943 | 104 |
| Nelfinavir | 0 | | 101 | | 86 | | 886 | | 1239 |
| | 2 | | 856 | | 846 | | 725 | | 770 |
| | 4 | | 992 | | 1526 | | 171 | | 544 |

20

3. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μ M.

To were similar to the determine if the cell uptake/metabolism is concentration dependent, selected PI's were incubated with the 1 mL of MT-2 cell suspension (2.74 mLn cells/mL) for 1 hr at 37°C at 3 different concentrations: 10, 5 and 1 μ M. Following incubation, cells were washed twice with the cell culture medium, lysed and assayed using HPLC with UV detection. The sample preparation and analysis ones described for MT-2 cells, quiescent and stimulated PBMC. Intracellular concentrations were calculated based on cell count, a published single cell volume of 0.338 pl for MT-2 cells, and concentrations of analytes in cell lysates. Data are shown in Table 6.

Uptake of all three selected PI's in MT-2 cells appears to be concentration-independent in the 1-10 μ M range. Metabolism (conversion to acid metabolites) appeared to be concentration-dependent for GS-16503 and GS-16577 (3-fold increase at 1 μ M vs. 10 μ M) but independent for GS-17394 (monolactate). Conversion from a respective metabolite X to GS-8373 was concentration-independent for both GS-16503 and GS-16577 (no conversion was observed for metabolite LX of GS-17394).

Table 6. Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μ M.

| Compound | Extracellular Concentration, μ M | Cell-Assosiated Prodrug and Metabolites Concentration, μ M | | | | % Conversion to acid metabolites |
|----------|--------------------------------------|--|--------|---------|-------|----------------------------------|
| | | Metabolite X | GS8373 | Prodrug | Total | |
| GS-17394 | 10 | 1358 | 0 | 635 | 1993 | 68 |
| | 5 | 916 | 0 | 449 | 1365 | 67 |
| | 1 | 196 | 0 | 63 | 260 | 76 |
| GS-16576 | 10 | 478 | 238 | 2519 | 3235 | 22 |
| | 5 | 250 | 148 | 621 | 1043 | 40 |
| | 1 | 65 | 36 | 61 | 168 | 64 |
| GS-16503 | 10 | 120 | 86 | 1506 | 1712 | 12 |
| | 5 | 58 | 60 | 579 | 697 | 17 |
| | 1 | 12 | 18 | 74 | 104 | 29 |

* For GS16576, Metabolite X is mono-aminobutyric acid

4. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10 μ M.

In order to estimate the relative intracellular levels of the different PI prodrugs under conditions simulating the in vivo environment, compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate (GS-16503), monoamidate phenoxy phosphonate (GS-16571) and monolactate phenoxy phosphonate (GS-17394) were incubated at 10 μ M for 1 hr with intact human whole blood at 37°C. After incubation, PBMC were isolated, then lysed and the lysates were analyzed by HPLC with UV detection. The results of analysis are shown in Table 7. There was significant cell uptake/metabolism following incubation in whole blood. There was no apparent difference in uptake between GS-16503 and GS-16571. GS-17394 demonstrated significantly higher intracellular levels than GS-16571 and GS-16503.

The degree of conversion to acid metabolites varies between different prodrugs after 1 hr incubation. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571 (Table 7). The metabolites, generally, were an equimolar mixture of the mono-phosphonic acid metabolite and GS-8373 (parent acid) except for GS-17394, where Metabolite LX was stable with no GS-8373 formed.

Table 7. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10 μ M (Mean \pm SD, N=3).

| GS# | Intracellular Prodrug and Metabolites Concentration, μ M | | | Major Intracellular Metabolites |
|-------|--|------------------|----------------|---------------------------------|
| | Acid Metabolite | Prodrug, μ M | Total, μ M | |
| 16503 | 279 \pm 47 | 61 \pm 40 | 340 \pm 35 | X , GS-8373 |
| 16571 | 319 \pm 112 | 137 \pm 62 | 432 \pm 208 | X, GS-8373 |
| 17394 | 629 \pm 303 | 69 \pm 85 | 698 \pm 301 | LX |

* PBMC Intracellular Volume = 0.2 μ L/mln

5. Distribution of PI Prodrugs in PBMC

5 In order to compare distribution and persistence of PI phosphonate prodrugs with those of non-prodrug PI's, GS-16503, GS-17394 and nelfinavir, were incubated at 10 μ M for 1 hr with PBMC (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 20 more hr (chase phase). At specific time points, the cells were washed and lysed. The cell cytosol was separated from membranes by centrifugation at 9000
10 xg. Both cytosol and membranes were extracted with acetonitrile and analyzed by HPLC with UV detection.

Table 8 shows the levels of total acid metabolites and corresponding prodrugs in the cytosol and membranes before and after the 22 hr chase. Both prodrugs exhibited complete
15 conversion to the acid metabolites (GS-8373 and X for GS-16503 and LX for GS-17394, respectively). The levels of the acid metabolites of the PI phosphonate prodrugs in the cytosol fraction were 2-3-fold greater than those in the membrane fraction after the 1 hr pulse and 10-fold greater after the 22 hr chase. Nelfinavir was present only in the membrane
fractions. The uptake of GS-17394 was about 3-fold greater than that of GS-16503 and 30-
20 fold greater than nelfinavir. The metabolites were an equimolar mixture of metabolite X and GS-8373 (parent acid) for GS-16503 and only metabolite LX for GS-17394.

Table 8. Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following Continuous and 1 hr Pulse/22 hr Chase Incubation of 10 μ M PI Prodrugs and Nelfinavir with Quiescent PBMC.

5

| GS# | Cell Type | Fraction | Cell-Associated PI, pmol/mln cells | | | |
|------------|-----------|----------|------------------------------------|---------|-------------------------|---------|
| | | | 1 hr Pulse/ 0 hr Chase | | 1 hr Pulse/ 22 hr Chase | |
| | | | Acid Metabolites | Prodrug | Acid Metabolites | Prodrug |
| GS-16503 | PBMC | Membrane | 228 | 0 | 9 | 0 |
| GS-16503 | PBMC | Cytosol | 390 | 0 | 130 | 0 |
| GS-17394 | PBMC | Membrane | 335 | 0 | 26 | 0 |
| GS-17394 | PBMC | Cytosol | 894 | 0 | 249 | 0 |
| Nelfinavir | PBMC | Membrane | | 42 | | 25 |
| Nelfinavir | PBMC | Cytosol | | 0 | | 0 |

Uptake and cell distribution of metabolites and intact prodrugs following 1 hr pulse/22 hr chase incubation of 10 μ M PI prodrugs and Nelfinavir with quiescent PBMC were measured.

10

6. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

The *in vitro* metabolism and stability of the PI phosphonate prodrugs were determined in PBMC extract, dog plasma and human serum (Table 9). Biological samples listed below (120 μ L) were transferred into an 8-tube strip placed in the aluminum 37°C heating block/holder and incubated at 37°C for 5 min. Aliquots (2.5 μ L) of solution containing 1 mM of test compounds in DMSO, were transferred to a clean 8-tube strip, placed in the aluminum 37°C heating block/holder. 60 μ L aliquots of 80% acetonitrile/20% water containing 7.5 μ M of amprenavir as an internal standard for HPLC analysis were placed into five 8-tube strips and kept on ice/refrigerated prior to use. An enzymatic reaction was started by adding 120 μ L aliquots of a biological sample to the strip with the test compounds using a multichannel pipet. The strip was immediately vortex-mixed and the reaction mixture (20 μ L) was sampled and transferred to the Internal Standard/ACN strip. The sample was considered the time-zero sample (actual time was 1-2 min). Then, at specific time points, the

reaction mixture (20 μ L) was sampled and transferred to the corresponding IS/ACN strip. Typical sampling times were 6, 20, 60 and 120 min. When all time points were sampled, an 80 μ L aliquot of water was added to each tube and strips were centrifuged for 30 min at 3000xG. The supernatants were analyzed with HPLC under the following conditions:

5

Column: Inertsil ODS-3, 75 x 4.6 mm, 3 μ m at 40°C.

Mobile Phase A: 20 mM ammonium acetate in 10%ACN/90%water

Mobile Phase B 20 mM ammonium acetate in 70%ACN/30%water

Gradient: 20% B to 100% B in 4 min, 2 min 100% B, 2 min 20% B

10 Flow Rate: 2 mL/min

Detection: UV at 243 nm

Run Time: 8 min

The biological samples evaluated were as follows:

15 PBMC cell extract was prepared from fresh cells using a modified published procedure (A. Pompon, I. Lefebvre, J-L. Imbach, S. Kahn, and D. Farquhar, Antiviral Chemistry & Chemotherapy, 5, 91 - 98 (1994)). Briefly, the extract was prepared as following: The cells were separated from their culture medium by centrifugation (1000 g, 15 min, ambient temperature). The residue (about 100 μ L, 3.5×10^8 cells) was resuspended in 4 mL of a
20 buffer (0.010 M HEPES, pH 7.4, 50 mM potassium chloride, 5 mM magnesium chloride and 5 mM dl-dithiothreitol) and sonicated. The lysate was centrifuged (9000 g, 10 min, 4°C) to remove membranes. The upper layer (0.5 mg protein/mL) was stored at -70°C. The reaction mixture contained the cell extract at about 0.5 mg protein/mL.

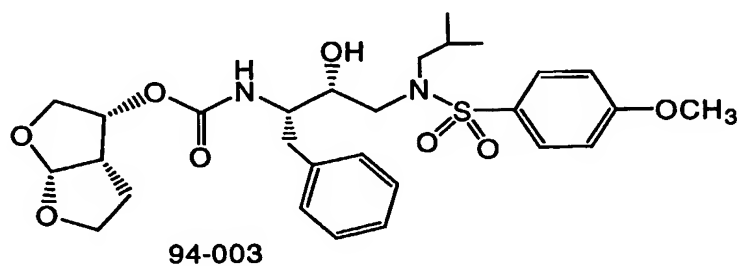
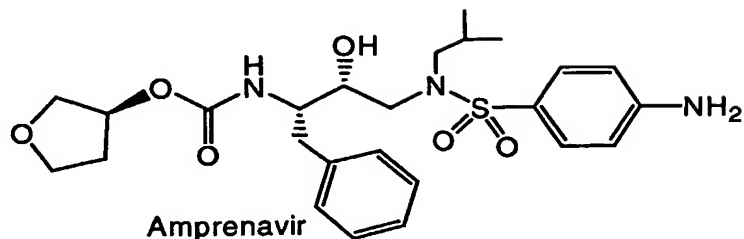
Human serum (pooled normal human serum from George King Biomedical Systems, Inc.).

25 Protein concentration in the reaction mixture was about 60 mg protein/mL.

Dog Plasma (pooled normal dog plasma (EDTA) from Pel Freez, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

Table 9: PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

| GS# | PBMC Extract ¹ T _{1/2} , min | Dog Plasma T _{1/2} , min | Human Serum T _{1/2} , min | HIV EC ₅₀ (nM) |
|-------|--|---|--|------------------------------|
| 16503 | 2 | 368 | >>400 | 6.0 ± 1.4 |
| 16571 | 49 | 126 | 110 | 15 ± 5 |
| 17394 | 15 | 144 | 49 | 20 ± 7 |

Example Section P**Table 10: Enzymatic and Cellular data****Formula II ALPPI activity****K_i [pM]**

| | | |
|----|------------------|-----|
| 10 | ≤ 10 | +++ |
| | > 10 to ≤ 100 | ++ |
| | > 100 to ≤ 1,000 | + |
| | > 1,000 | - |

EC₅₀ [nM]

| | | |
|----|------------------|-----|
| 15 | ≤ 50 | +++ |
| | > 50 to ≤ 500 | ++ |
| | > 500 to ≤ 5,000 | + |
| | > 5,000 | - |

I50V and I84V/L90M fold change

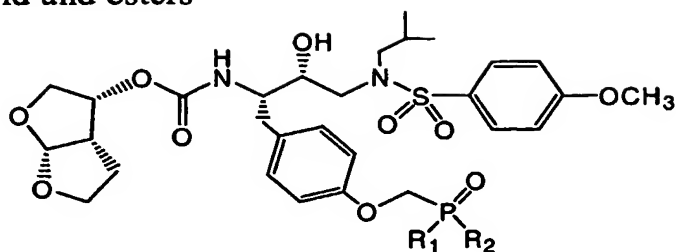
| | | |
|----|--------------|-----|
| 20 | > 30 | +++ |
| | > 10 to ≤ 30 | ++ |
| | > 3 to ≤ 10 | + |
| 25 | ≤ 3 | - |

CC₅₀ [μM]

| | | |
|----|-------------|----|
| 30 | ≤ 5 | ++ |
| | > 5 to ≤ 50 | + |
| | > 50 | - |

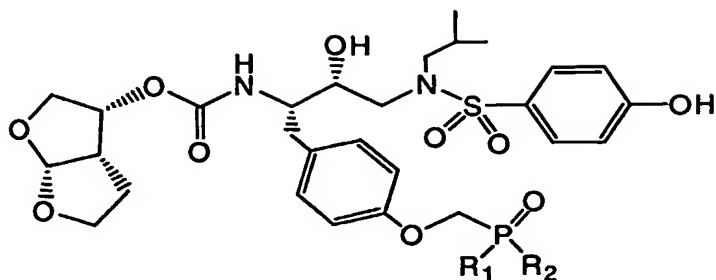
| Compound | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I50V (#2) fold change | I84V/L90 M fold change | CC ₅₀ (μM) |
|------------|------------|--------------------------|--------------------------|--------------------------------|---------------------------------|--------------------------|
| Saquinavir | ++ | +++ | — | — | +++ | |
| Nelfinavir | + | +++ | — | + | +++ | |
| Indinavir | + | +++ | — | + | +++ | |
| Ritonavir | ++ | +++ | ++ | ++ | +++ | |
| Lopinavir | ++ | +++ | ++ | +++ | ++ | |
| Amprenavir | + | +++ | +++ | +++ | ++ | — |
| Atazanavir | ++ | +++ | — | — | +++ | |
| Tipranavir | ++ | ++ | — | — | + | |
| 94-003 | +++ | +++ | +++ | +++ | ++ | + |
| TMC114 | +++ | +++ | ++ | ++ | — | |

P1-Phosphonic acid and esters



| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ (μM) |
|----------------------------------|----------------------------------|------------|--------------------------|--------------------------|--------------------------|-----------------------|
| OH | OH | +++ | + | - | - | - |
| OMe | OMe | ++ | +++ | | | |
| OEt | OEt | +++ | +++ | - | - | + |
| OCH ₂ CF ₃ | OCH ₂ CF ₃ | ++ | - | | | |
| OiPr | OiPr | ++ | +++ | - | - | |
| OPh | OPh | | +++ | | | |
| OMe | OPh | ++ | +++ | | | |
| OEt | OPh | +++ | +++ | | | |
| OBn | OBn | ++ | +++ | - | - | + |
| OEt | OBn | ++ | +++ | | | ++ |
| OPoc | OPoc | | + | | | |
| OH | OEt | | ++ | | | |
| OH | OPh | +++ | - | | | |
| OH | OBn | | + | - | - | |

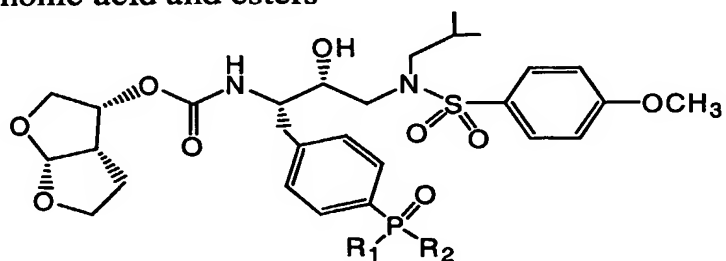
P1-Phosphonic acid and esters



5

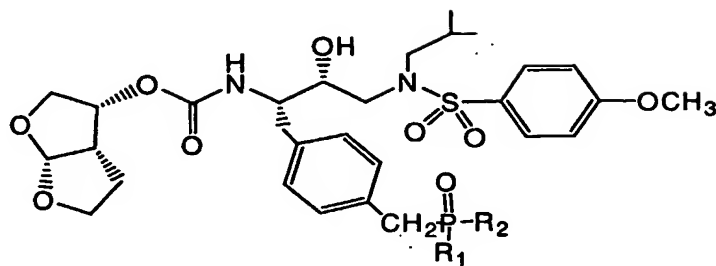
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ (μM) |
|----|----|------------|--------------------------|--------------------------|--------------------------|-----------------------|
| OH | OH | +++ | + | | | |
| Et | Et | +++ | +++ | | | |

P1-Direct phosphonic acid and esters



10

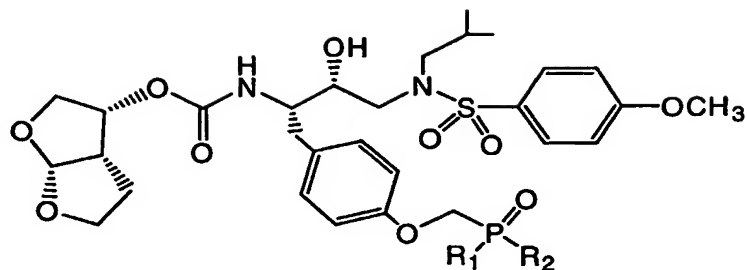
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-----|-----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OH | OH | ++ | - | | | |
| OEt | OEt | +++ | +++ | + | - | |

P1-CH₂-phosphonic acid and esters

5

| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OE | OE | +++ | +++ | + | + | |

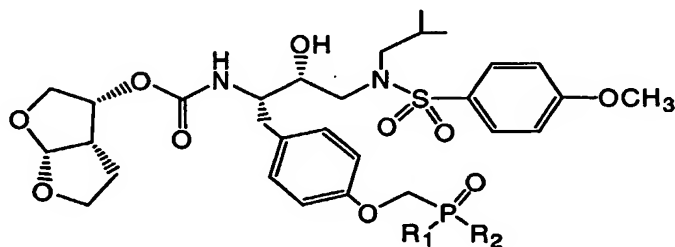
P1-P-Bisamidates



| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|--------|--------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| NHEt | NHEt | +++ | ++ | — | — | |
| Gly-Et | Gly-Et | ++ | ++ | | | |
| Gly-Bu | Gly-Bu | +++ | +++ | | | |
| Ala-Et | Ala-Et | ++ | ++ | | — | — |
| Ala-Bu | Ala-Bu | ++ | +++ | + | — | |
| Aba-Et | Aba-Et | +++ | +++ | | | |
| Aba-Bu | Aba-Bu | +++ | +++ | ++ | + | |
| Val-Et | Val-Et | + | +++ | — | — | |
| Leu-Et | Leu-Et | ++ | +++ | | | |
| Leu-Bu | Leu-Bu | ++ | ++ | + | + | |
| Phe-Et | Phe-Et | | +++ | | | |
| Phe-Bu | Phe-Bu | | +++ | | | |

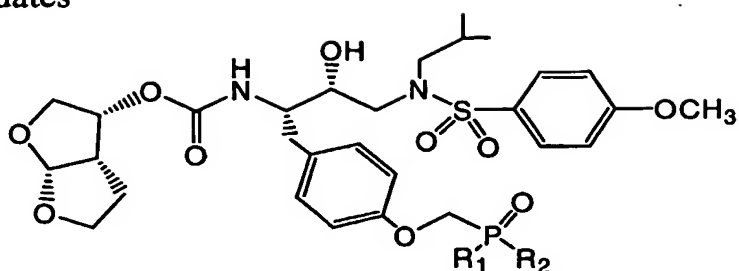
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P1-P-Bislactates



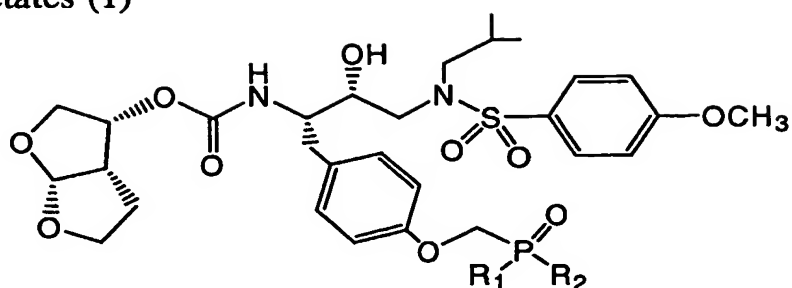
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|---------|---------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| Glc-Et | Glc-Et | +++ | + | — | — | |
| Lac-Et | Lac-Et | ++ | ++ | — | — | |
| Lac-iPr | Lac-iPr | ++ | +++ | | — | |

P1-P-Monoamidates



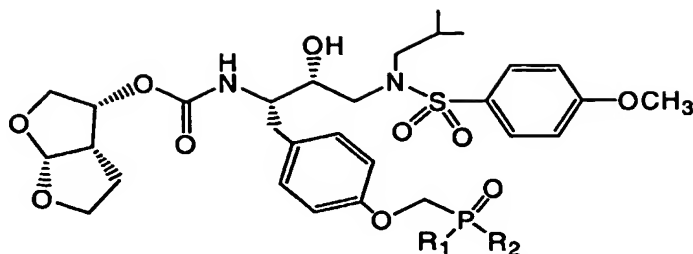
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-----|------------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh | Gly-Bu | ++ | ++ | - | - | |
| OPh | Ala-Me | ++ | +++ | | - | |
| OPh | Ala-Et | +++ | +++ | - | - | |
| OPh | Ala-iPr | ++ | +++ | - | - | |
| OPh | Ala-iPr | +++ | +++ | | | |
| OPh | Ala-iPr | ++ | +++ | | | |
| OPh | (D)Ala-iPr | ++ | +++ | | - | |
| OPh | (D)Ala-iPr | +++ | +++ | | | |
| OPh | (D)Ala-iPr | +++ | +++ | | | |
| OPh | Ala-Bu | ++ | +++ | - | - | |
| OPh | Ala-Bu | ++ | +++ | - | | |
| OPh | Ala-Bu | ++ | +++ | - | | |
| OPh | Aba-Et | | +++ | | | |
| OPh | Aba-Et | | +++ | - | - | |
| OPh | Aba-Et | | ++ | | | |
| OPh | Aba-Bu | | +++ | + | - | |
| OPh | Aba-Bu | | ++ | - | - | |
| OBn | Ala-Et | +++ | +++ | - | - | |
| OH | Ala-OH | +++ | - | | | |
| OH | Ala-Bu | | - | | | |

P1-P-Monolactates (1)



| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I50V (#2) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------------------------|-----------|------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh | Glc-Et | +++ | +++ | – | | – | |
| OPh | Lac-Me | | ++ | – | | | |
| OPh | Lac-Et | | +++ | – | + | – | + |
| OPh | Lac-Et | +++ | +++ | – | | – | |
| OPh | Lac-Et | ++ | +++ | – | | – | |
| OPh | Lac-iPr | ++ | +++ | – | | – | |
| OPh | Lac-iPr | +++ | +++ | | | | |
| OPh | Lac-iPr | ++ | +++ | | | | |
| OPh | Lac-Bu | ++ | ++ | | | – | |
| OPh | Lac-Bu | ++ | ++ | | | | |
| OPh | Lac-Bu | ++ | ++ | | | | |
| OPh | Lac-EtMor | | – | | | | |
| OPh | Lac-PrMor | | – | | | | |
| OPh | (R)Lac-Me | +++ | +++ | | | | |
| OPh | (R)Lac-Et | +++ | +++ | – | | – | |
| OEt | Lac-Et | | ++ | | | | |
| OCH ₂ CF ₃ | Lac-Et | | ++ | | | | |
| OBn | Lac-Bn | ++ | ++ | | | | |
| OBn | (R)Lac-Bn | | | | | | |
| OH | Lac-OH | +++ | + | | | – | |
| OH | (R)Lac-OH | ++ | + | | | – | |

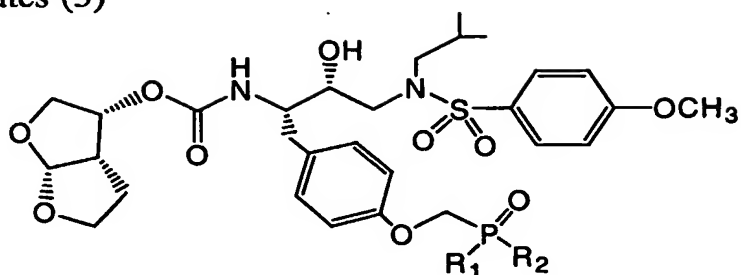
P1-P-Monolactates (2)



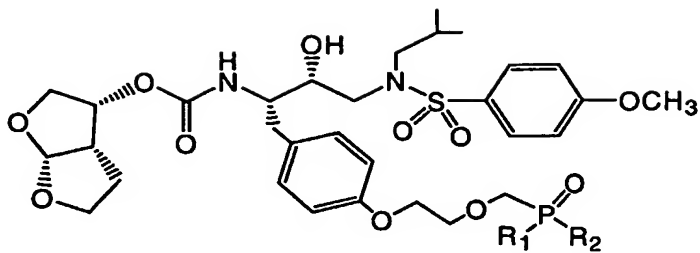
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| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-----|--------------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| OPh | mix-Hba-Et | ++ | +++ | + | — | |
| OPh | (S)Hba-Et | + | +++ | | | |
| OPh | (S)Hba-tBu | | +++ | | | |
| OH | (S)Hba-OH | ++ | | | | |
| OPh | (R)Hba-Et | | +++ | | | |
| OPh | (S)MeBut-Et | | +++ | | | |
| OPh | (R)MeBut-Et | | +++ | | | |
| OPh | DiMePro-Me | ++ | | | | |
| OPh | (S)Lac-EtMor | | — | | | |
| OPh | (S)Lac-PrMor | | — | | | |
| OPh | (S)Lac-EtPip | | ++ | — | — | |

P1-P-Monolactates (3)

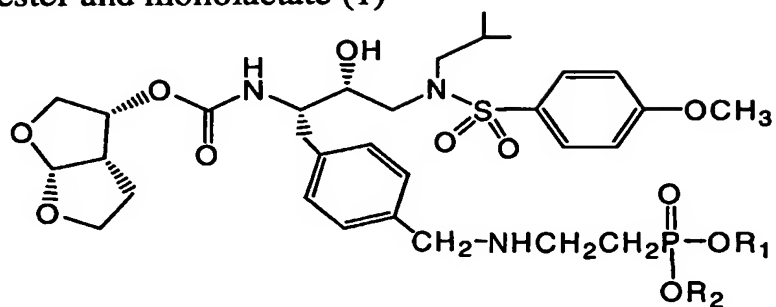


| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|---|-----------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh— <i>o</i> -i-But | (S)Lac-Et | | +++ | | | |
| OPh— <i>p</i> -n-Oct | (S)Lac-Et | | ++ | | | |
| OPh— <i>p</i> -n-But | (S)Lac-Et | | +++ | | | |
| OPh— <i>m</i> -COOBn | (S)Lac-Et | | ++ | | | |
| OPh— <i>m</i> -COOH | (S)Lac-Et | | ++ | | | |
| OPh— <i>m</i> -CH ₂ OH | (S)Lac-Et | | ++ | — | — | |
| OPh— <i>m</i> -CH ₂ NH ₂ | (S)Lac-Et | ++ | ++ | | | |
| OPh— <i>m</i> -CH ₂ NMe ₂ | (S)Lac-Et | | + | | | |
| OPh— <i>m</i> -CH ₂ Mor | (S)Lac-Et | | ++ | — | — | |
| OPh— <i>m</i> -CH ₂ Pip | (S)Lac-Et | | ++ | | | |
| OPh— <i>m</i> -CH ₂ NMeC ₂ OM | (S)Lac-Et | | ++ | | | |
| OPh— <i>o</i> -OEt | (S)Lac-Et | | +++ | | | |
| ONMe ₂ | (S)Lac-Et | | ++ | | | |
| OPip | (S)Lac-Et | | + | | | |
| OMor | (S)Lac-Et | | — | | | |

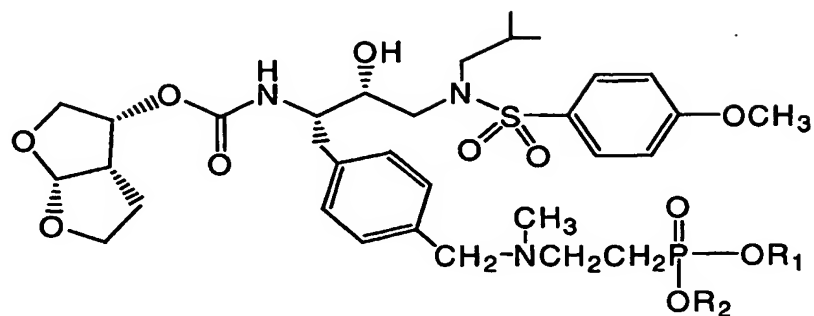
P1-C₂H₄-P-Monolactates

5

| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-------------------------------------|--------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| -OC ₂ H ₄ OBn | | | +++ | | | |
| OEt | OEt | | +++ | - | - | |
| OPh | Lac-Et | | ++ | - | - | |
| OH | OH | ++ | | | | |
| OH | Lac | ++ | | | | |

P1-CH₂N-P-diester and monolactate (1)

| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I50V (#2) fold change | I84V/L9M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|-----------------------------|--------------------------|----------------------------|------------------------|
| Et | Et | ++ | +++ | | — | | |
| H | H | ++ | - | | + | | |
| Ph | Lac-Et | | ++ | — | ++ | — | |
| Ph | Lac-Et | | + | | + | — | — |
| Ph | Lac-Et | | + | | ++ | — | |
| Ph | Aba-Et | | + | | + | — | |
| Ph- oEt | Lac-Et | ++ | ++ | — | ++ | — | |
| Ph- dM | Lac-Et | | +++ | | + | + | |
| Ph- dM | Lac-Pr | | +++ | | | | |
| H | Lac | ++ | | | | | |
| Ph | Hba-Et | | ++ | | ++ | — | |
| Ph | Hba-Et | | ++ | | ++ | — | + |
| Ph | Hba-Et | | ++ | | ++ | — | |
| H | Hba | + | | | | | |

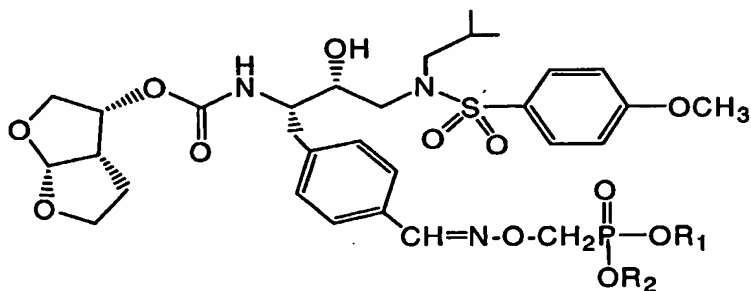
P1-CH₂N-P-diester and monolactate (2)

5

| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| Ph | Lac-Et | + | ++ | + | + | |
| H | H | ++ | | | | |

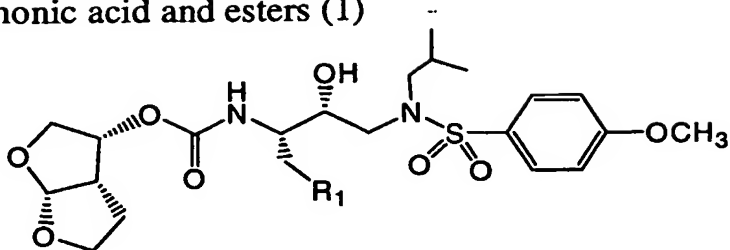
P1-CH₂N-P-diester and monolactate (3)

10



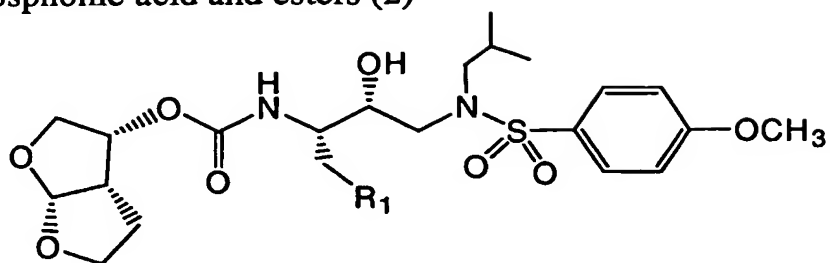
| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| Et | Et | ++ | +++ | | - | |

P1-N-P1-Phosphonic acid and esters (1)



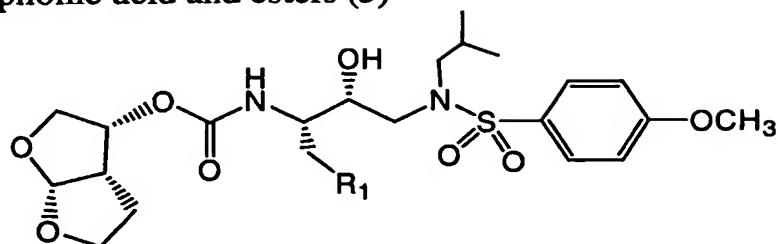
| R1 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|------------|--------------------------|-----------------------------|--------------------------|---------------------|
| | - | ++ | | | |
| | - | ++ | | | |
| | - | | | | |
| | ++ | +++ | | + | |
| | | - | | | |
| | - | | | | |
| | + | ++ | | | |
| | ++ | +++ | | + | |
| | | - | | | |
| | | - | | | |
| | - | | | | |
| | + | +++ | | + | |

P1-N-P1-Phosphonic acid and esters (2)



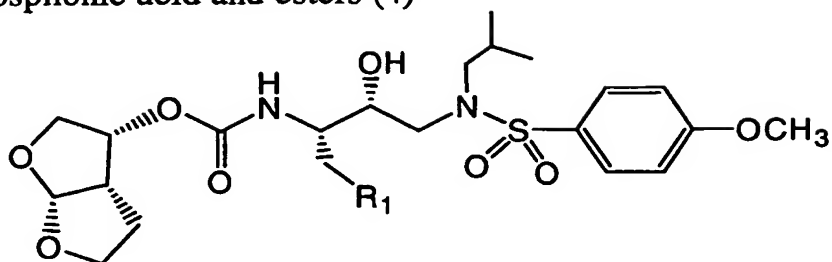
| R1 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | + | + | | + | |
| | ++ | +++ | | + | |
| | ++ | +++ | | | |
| | ++ | ++ | | - | |
| | | +++ | | | |
| | ++ | +++ | | + | |
| | | +++ | | - | |
| | - | +++ | | ++ | |
| | - | | | | |
| | + | +++ | +++ | - | |
| | - | | | | |
| | | +++ | ++ | + | |
| | - | | | | |

P1-N-P1-Phosphonic acid and esters (3)



| R1 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | ++ | +++ | + | + | |
| | + | ++ | + | + | |
| | + | ++ | + | + | |
| | + | | | | |
| | | | | | |
| | - | - | | | |

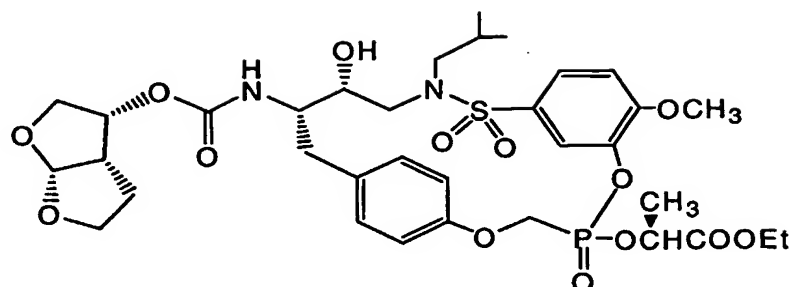
P1-N-P1-Phosphonic acid and esters (4)



| R1 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | +++ | | | | |
| | +++ | +++ | - | - | |
| | ++ | +++ | + | - | |
| | ++ | +++ | | | |
| | ++ | ++ | | | |
| | +++ | +++ | | | |
| | | +++ | ++ | - | |
| | | +++ | ++ | - | |
| | ++ | | | | |
| | ++ | | | | |

P1- P-cyclic monolactate

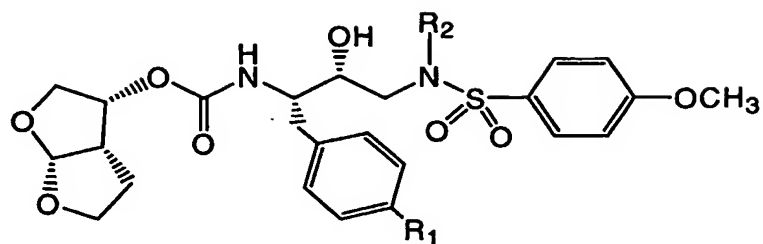
5



| R ₁ | R ₂ | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | nd | nd | | | |
| | | nd | nd | | | |

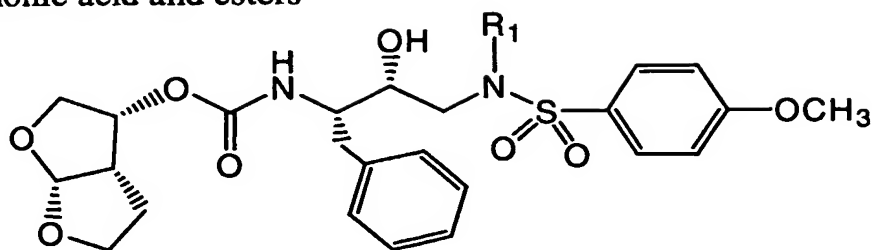
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P1'-N-P1-Phosphonic acid and esters



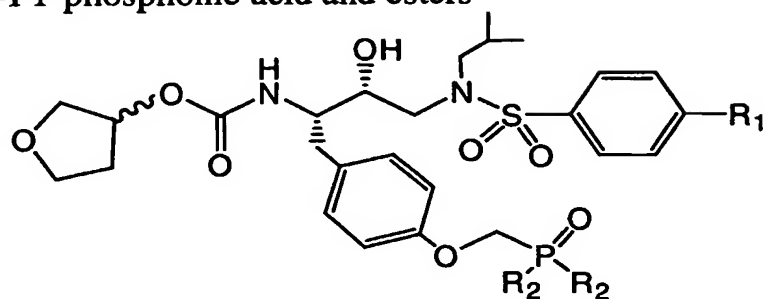
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|--------------------|----|---------|-----------------------|-----------------------|-----------------------|---------------------|
| CH ₃ | | ++ | +++ | ++ | + | |
| OH | | | +++ | - | - | |
| CH ₂ OH | | +++ | +++ | - | - | |
| OBn | | +++ | +++ | - | - | |
| OH | | - | ++ | - | - | |
| OBn | | - | +++ | | - | |
| | | - | - | + | + | |
| | | + | ++ | + | + | |
| OH | | - | - | | | |
| | | ++ | - | | | |
| | | ++ | - | | | |
| | | ++ | ++ | | | |
| | | + | - | | | |

P1'-Phosphonic acid and esters



| R1 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | ++ | +++ | +++ | +++ | |
| | +++ | +++ | +++ | +++ | |
| | ++ | + | | +++ | |
| | +++ | +++ | | +++ | |
| | +++ | +++ | | ++ | |
| | ++ | ++ | ++ | ++ | |
| | ++ | +++ | +++ | +++ | |

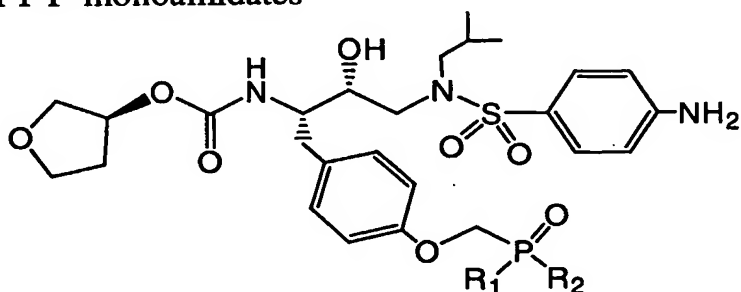
P2-Monofuran-P1-phosphonic acid and esters



| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-----------------|--------|---------|-----------------------|-----------------------|-----------------------|---------------------|
| OMe | OH | | - | +++ | +++ | |
| OMe | OEt | +++ | +++ | +++ | ++ | |
| OMe | OBn | | +++ | ++ | ++ | |
| OMe | phenol | +++ | +++ | +++ | + | |
| OMe | OEt | ++ | +++ | +++ | ++ | |
| NH ₂ | phenol | + | ++ | + | - | |
| NH ₂ | OH | | - | | + | |
| NH ₂ | OBn | ++ | ++ | | + | |

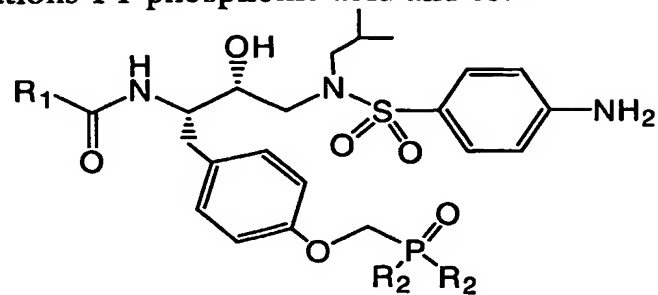
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P2-Monofuran-P1-P-monoamidates



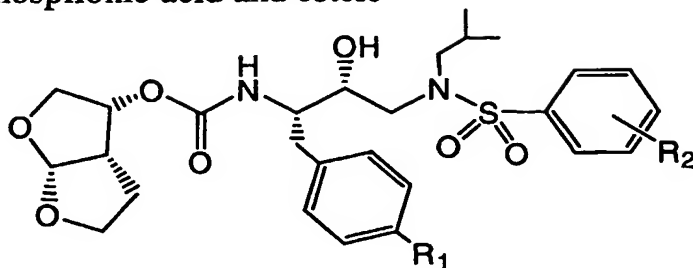
| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|-----|---------|---------|-----------------------|-----------------------|-----------------------|---------------------|
| OPh | Ala-iPr | ++ | ++ | | + | |
| OPh | Ala-iPr | ++ | ++ | | | |
| OPh | Ala-iPr | + | ++ | | | |

P2-Other modifications-P1-phosphonic acid and esters



| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|--------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| | phenyl | + | +++ | +++ | ++ | |
| | phenol | + | ++ | ++ | + | |
| | OH | - | - | ++ | - | |
| | OBn | + | ++ | + | - | |
| | phenyl | + | ++ | +++ | + | |
| | OH | + | - | ++ | + | |
| | OBn | + | ++ | +++ | + | |
| | phenyl | - | ++ | | ++ | |
| | phenol | + | + | | - | |
| | OH | + | - | - | - | |
| | OBn | ++ | ++ | + | - | |

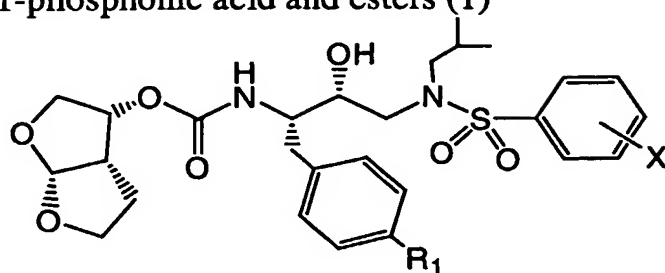
P2'-Amino-P1-phosphonic acid and esters



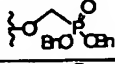
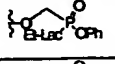
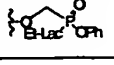
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| R1 | R2 | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|-----------------------------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OH | <i>p</i> -NH ₂ | ++ | ++ | - | - | |
| | <i>p</i> -NH ₂ | ++ | - | + | - | |
| | <i>p</i> -NH ₂ | ++ | +++ | | - | |
| | <i>p</i> -NO ₂ | ++ | +++ | | - | |
| | <i>p</i> -NHEt ₁ | ++ | +++ | | - | |
| | <i>p</i> -NH ₂ | ++ | +++ | - | - | |
| OH | <i>m</i> -NH ₂ | ++ | ++ | | - | |
| | <i>m</i> -NH ₂ | ++ | + | | - | |
| | <i>m</i> -NH ₂ | ++ | ++ | | - | |
| | <i>m</i> -NH ₂ | ++ | +++ | - | - | |
| | <i>m</i> -NH ₂ | + | ++ | - | - | |
| | <i>m</i> -NH ₂ | ++ | ++ | | | |
| | <i>m</i> -NH ₂ | + | ++ | | | |

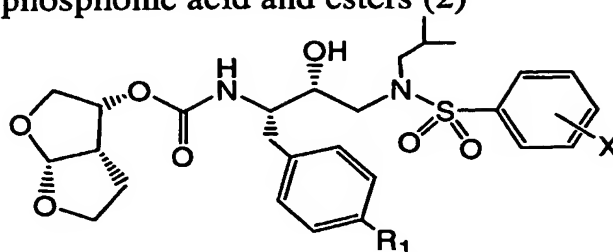
P2'-Substituted-P1-phosphonic acid and esters (1)



| R1 | X | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|----------------------------|---------|-----------------------|-----------------------|-----------------------|---------------------|
| | <i>p</i> -OH | +++ | + | | | |
| | <i>p</i> -OH | +++ | +++ | | | |
| | <i>p</i> -OH | ++ | | | | |
| | <i>p</i> -OH | | +++ | | - | |
| | <i>p</i> -OBn | | ++ | | | |
| | <i>p</i> -OBn | | - | | | |
| | <i>p</i> -H | ++ | - | | | |
| | <i>p</i> -H | ++ | +++ | | + | |
| | <i>p</i> -H | | +++ | + | + | |
| | <i>p</i> -H | | ++ | | | |
| | <i>p</i> -H | ++ | | | | |
| | <i>p</i> -F | ++ | + | | | |
| | <i>p</i> -F | ++ | +++ | | + | |
| | <i>p</i> -F | | +++ | + | + | |
| | <i>p</i> -F | | ++ | + | + | |
| | <i>p</i> -F | ++ | | | | |
| | <i>p</i> -CF ₃ | +++ | + | | | |
| | <i>p</i> -CF ₃ | ++ | +++ | | - | |
| | <i>p</i> -OCF ₃ | ++ | + | | | |
| | <i>p</i> -OCF ₃ | ++ | +++ | | + | |

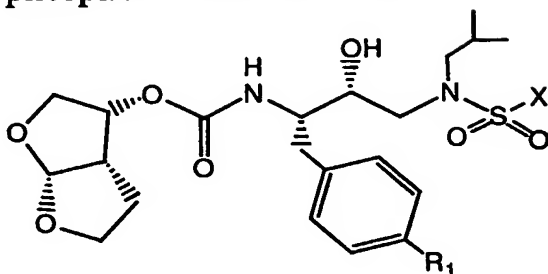
| | | | | | | |
|---|----------------------|----|-----|--|---|--|
|  | <i>p</i> -CN | ++ | +++ | | - | |
|  | <i>p</i> -Pip | - | - | | | |
|  | <i>p</i> -Pip- Me | - | - | | | |

P2'-Substituted-P1-phosphonic acid and esters (2)



| R1 | X | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|--------------------|-------------------------------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| | <i>m</i> -Py | ++ | +++ | | | |
| | <i>m</i> -Py | ++ | | | | |
| | <i>m</i> -Py | ++ | ++ | + | - | |
| | <i>m</i> -Py | ++ | ++ | | | |
| | <i>m</i> -Py | ++ | | | | |
| | <i>m</i> -Py-Me ⁺ | | + | | | |
| | <i>m</i> -Py-Me ⁺ | | ++ | | | |
| | <i>m</i> -Py-oxide | | ++ | | | |
| | <i>m</i> -Py-oxide | ++ | | | | |
| | <i>m</i> -Py-oxide | ++ | ++ | | - | |
| | <i>m</i> -Py-oxide | + | | | | |
| | <i>m</i> -Py-oxide | | - | | | |
| <i>p</i> -Py-oxide | <i>p</i> -OMe | ++ | - | | | |
| | <i>p</i> -CHO | | +++ | | | |
| | <i>p</i> -CHO | | +++ | | | |
| | <i>p</i> -CH ₂ OH | | +++ | - | - | |
| | <i>p</i> -CH ₂ OH | ++ | | | | |
| | <i>p</i> -CH ₂ OH | ++ | | | | |
| | <i>p</i> -CH ₂ Mor | | ++ | - | - | |
| | <i>p</i> -CH ₂ Mor | - | | | | |
| | <i>p</i> -CH ₂ Mor | - | | | | |

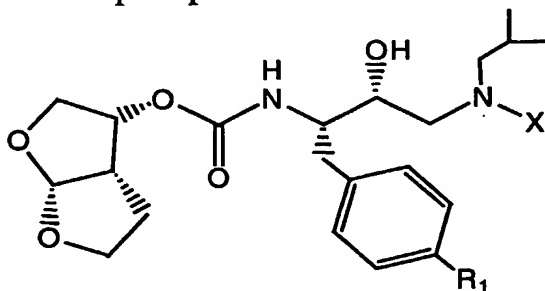
P2'-Alkylsulfonyl-P1-phosphonic acid and esters



| R1 | X | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|---|------------|--------------------------|--------------------------|--------------------------|------------------------|
| | | - | - | | | |
| | | + | ++ | | | |

5

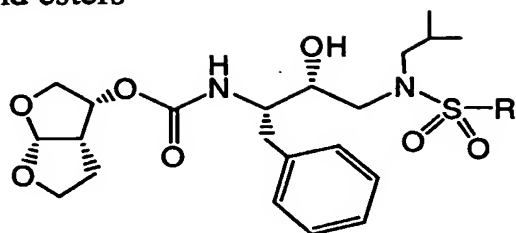
P2'-Carbonyl-substituted-P1-phosphonic acid and esters



| R1 | X | Ki (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----|---|------------|--------------------------|--------------------------|--------------------------|------------------------|
| | | - | | | | |
| | | - | ++ | | | |
| | | | + | | | |

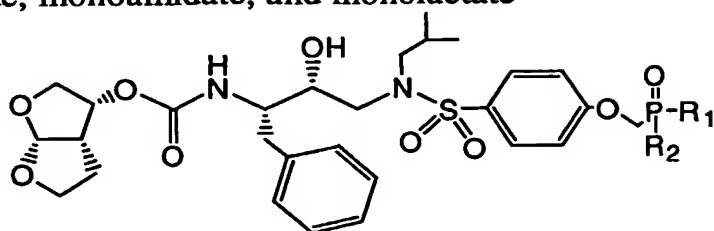
10

P2'-Phosphonic acid and esters



| R | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|---|------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| | +++ | +++ | - | - | |
| | +++ | + | - | - | |
| | ++ | - | | | |
| | ++ | +++ | ++ | ++ | |
| | + | ++ | +++ | +++ | |
| | +++ | +++ | + | + | |
| | +++ | +++ | +++ | ++ | |
| | ++ | ++ | ++ | + | |
| | +++ | +++ | +++ | ++ | |
| | ++ | +++ | ++ | ++ | |
| | +++ | +++ | - | - | |
| | +++ | ++ | + | - | |
| | + | ++ | + | + | |
| | - | + | +++ | ++ | |
| | + | ++ | + | - | |

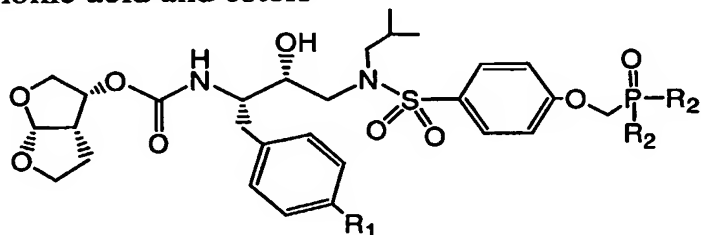
P2'-P-Bisamidate, monoamidate, and monolactate



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| Ala-Bu | Ala-Bu | + | ++ | + | + | |
| OPh | Ala-iPr | ++ | ++ | | | |
| OPh | Lac-iPr | + | + | | | |
| OH | Ala-OH | ++ | | | | |

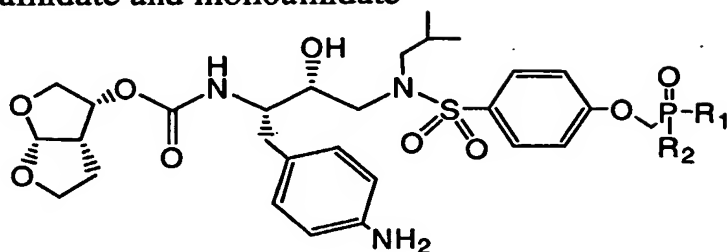
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P1-N-P2'-Phosphonic acid and esters



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|--|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| NO ₂ | phenol | | +++ | - | | |
| NH ₂ | OH | ++ | - | | | |
| NH ₂ | OEt | + | ++ | | ++ | |
| NH ₂ | OBn | + | + | | + | |
| NMe ₂ | OEt | ++ | +++ | | ++ | |
| OH | OH | ++ | - | | | |
| OH | OBn | ++ | ++ | | | |
| OC ₂ H ₄ NMe ₂ | OH | +++ | + | | | |
| OC ₂ H ₄ -NMe ₂ | OBn | ++ | ++ | | | |

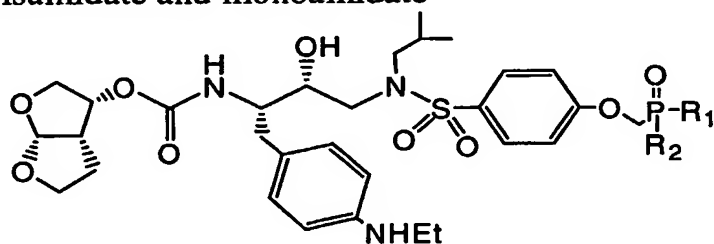
P1-N-P2'-P-Bisamidate and monoamidate



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| Ala-Bu | Ala-Bu | + | + | | | |
| OPh | Ala-iPr | + | - | | | |
| OPh | Ala-iPr | ++ | - | | | |

5

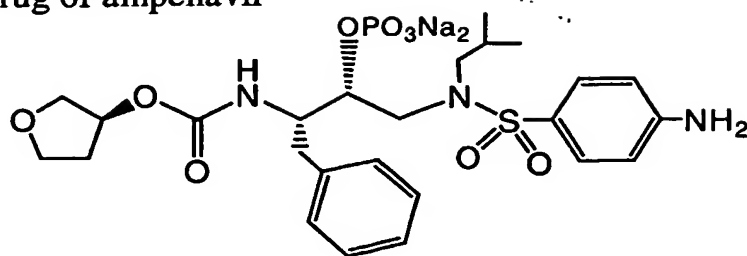
P1-NEt-P2'-P-Bisamidate and monoamidate



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh | Ala-iPr | + | + | | | |
| OPh | Ala-iPr | + | + | - | - | |

10

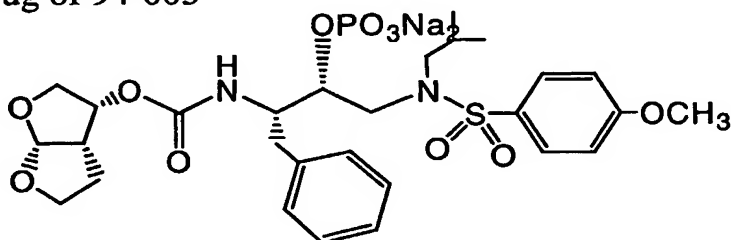
Phosphate prodrug of ampenavir



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | | ++ | | | |

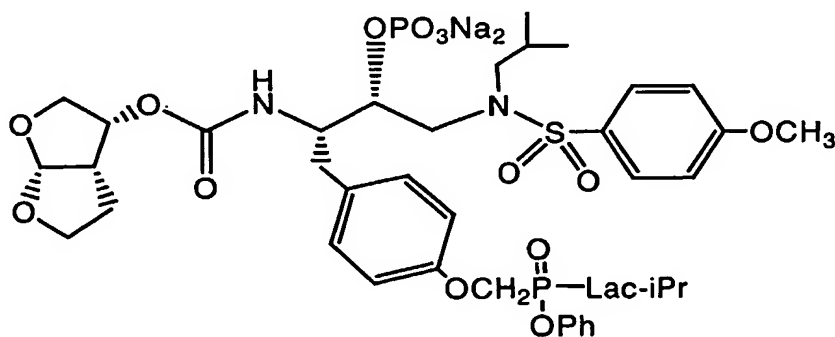
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Phosphate prodrug of 94-003



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | | +++ | | | |

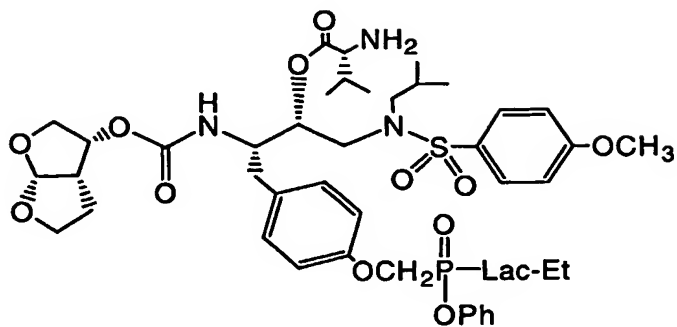
10 Phosphate prodrug of GS77366 (P1-mono(S)Lac-iPr)



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | | +++ | | | |

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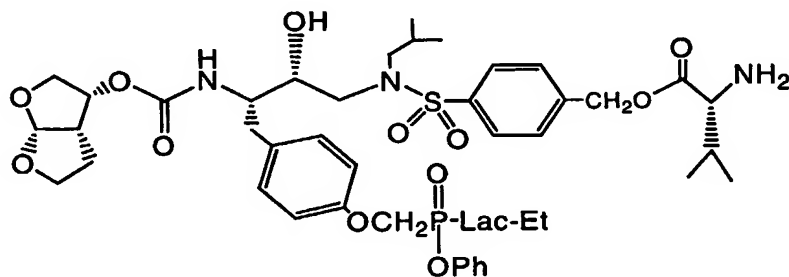
Valine prodrug of (P1-mono(S)Lac-Et).



| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | | ++ | | | |

5

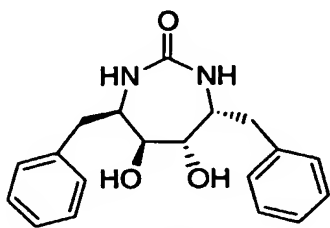
Valine prodrug of GS278053 (P1-mono(S)Lac-Et,P2'-CH₂OH)



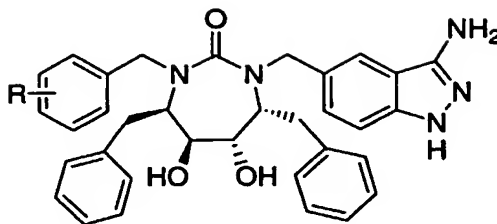
| R ₁ | R ₂ | K _i (pM) | EC ₅₀ (nM) | I50V (#1) fold change | I84V/L90M fold change | CC ₅₀ μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | | ++ | | | |

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Table 11: Enzymatic and Cellular Activity Data

Formula VIIIa CCLPPI activity

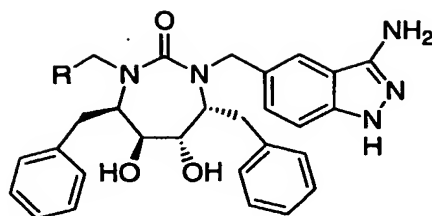
DMP-850



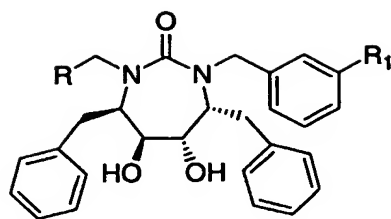
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| Structure, R | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|--|------------------------|--------------------------------|--|---|------------|-------------------|-------------------|-------------------|-------------------|------------|
| | K _i (nM) | WT IC ₅₀ / nM | 84V9 0M IC ₅₀ / nM | WT | 84V9 0M | 30N 82I88 D | 48V5 4V82 A | 48V5 4V82 S | 48V8 2A90 M | 46I50 V |
| H (DMP-850) | 0.033 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| p-OH | 0.029 | 3.0 | 12 | 149 | 143 | 79 | 32 | 39 | 19 | 55 |
| p-OBn | >5 | 353 | 781 | 2123 | 5312 | 1548 | ND | ND | ND | ND |
| p-OCH ₂ PO ₃ Bn ₂ | >5 | 276 | 2042 | 2697 | 4963 | 2119 | ND | ND | ND | ND |
| p-OCH ₂ PO ₃ Et ₂ | >5 | 627 | 1474 | 2480 | >600 0 | 1340 | ND | ND | ND | ND |
| p-OCH ₂ PO ₃ H ₂ | >5 | 551 | 1657 | >1200 0 | ND | ND | ND | ND | ND | ND |
| m-OH | 0.128 | 1.6 | 12 | 151 | 475 | 249 | 84 | | | 104 |
| m-OBn | 0.253 | 6.9 | 27 | 218 | 2422 | 82 | 709 | ND | ND | 601 |
| m-OCH ₂ PO ₃ Bn ₂ (N-iPr indazole) | 1.54 ^a | 31 | 72 | 489 | 514 | 237 | 159 | 171 | 168 | 708 |
| m-OCH ₂ PO ₃ Bn ₂ | 0.177 | 18 | 43 | 898 | >600 0 | 705 | 2597 | ND | ND | 3121 |
| m-OCH ₂ PO ₃ Et ₂ | 1.93 ^a | 70 | 169 | 665 | 3005 | 93 | 513 | ND | ND | 857 |
| m-OCH ₂ PO ₃ H ₂ | 0.254 | 8.3 | 33 | >1200 0 | ND | ND | ND | ND | ND | ND |

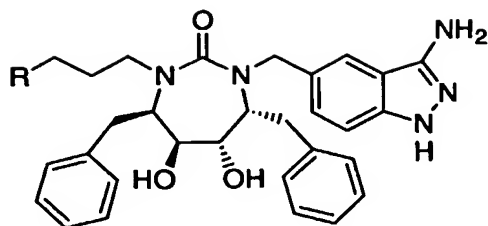
| | | | | | | | | | | |
|--|--------------------|-----|-----|------|-----------|------|------|----|----|-----------|
| m-OCH ₂ PO ₃ Ph ₂ | 0.543 _a | 10 | 42 | 1349 | >600 0 | 1541 | 2183 | ND | ND | 3380 |
| m-OCH ₂ PO ₃ HPh | 0.644 | 17 | 65 | 1745 | >600 0 | ND | ND | ND | ND | ND |
| m-mono-Ala-Bu | 0.858 _a | 6.6 | 39 | 1042 | >600 0 | 425 | 790 | ND | ND | 797 |
| m-mono-Ala-Et ^f | | 35 | 68 | 1436 | >600 0 | 219 | 734 | ND | ND | 1350 |
| m-mono-Lac-Bu | | 15 | 34 | 2663 | >600 0 | 1089 | ND | ND | ND | ND |
| m-mono-Lac-Et | | 23 | 80 | 2609 | >600 0 | 516 | 5923 | ND | ND | >600 0 |
| m-bis-Ala-Bu | 1.279 _a | 18 | 103 | 1079 | >600 0 | 2362 | 1854 | ND | ND | 1536 |
| m-bis-Ala-Et | 1.987 _a | 31 | 202 | 5620 | >600 0 | 1852 | ND | ND | ND | ND |



| Structure, R | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ /nM | | | | | | |
|----------------|------------------------|-------------------------------|------------------------------------|--|--------|-------------------|-------------------|-------------------|-------------------|------------|
| | K _i (nM) | WT IC ₅₀ /nM | 84V90 M IC ₅₀ /nM | WT | 84V90M | 30N 82188 D | 48V5 4V82 A | 48V5 4V82 S | 48V8 2A90 M | 46I50 V |
| H (DMP-850) | 0.033 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| | 0.091 | 3.4 | 27 | 1548 | >6000 | >6000 | ND | ND | ND | ND |
| | 0.354 | 3.3 | 25 | 168 | 909 | 750 | 277 | | | 489 |
| | 0.157 | 1.6 | 10 | 188 | 476 | 666 | 240 | | | 319 |
| | 0.044 | 5.0 | 27 | 491 | 387 | 234 | 238 | | | 192 |
| | 0.362 | 7.3 | 70 | 5141 | >6000 | 4480 | ND | ND | ND | ND |
| | 0.112 | 1.4 | 6.4 | 603 | 1276 | 678 | 208 | | | 209 |
| | <0.03 | 1.3 | 7.5 | 625 | 708 | 899 | 301 | | | 398 |

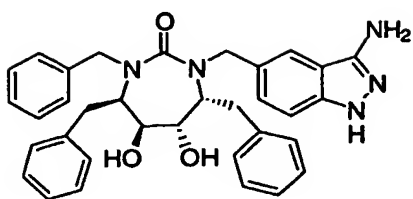


| Structure, R1 | Structure, R | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|---|--------------|------------------------|--------------------------------|--|---|------------|-------------------|-------------------|-------------------|-------------------|------------|
| | | K _i (nM) | WT IC ₅₀ / nM | 84V 90M IC ₅₀ / nM | WT | 84V 90M | 30N 82I8 8D | 48V 54V 82A | 48V 54V 82S | 48V 82A 90M | 46I5 0V |
| CO ₂ H | | | 15 | 174 | 3055 | >600 0 | 887 | ND | ND | ND | ND |
| CONH(CH ₂) ₃ PO ₃ Et ₂ | | 0.00 9 | 1.1 | 12 | 65 | 311 | 74 | 80 | 75 | 74 | 85 |
| CO ₂ H | | | 18 | 299 | 2344 | >600 0 | 3360 | ND | ND | ND | ND |
| CONH(CH ₂) ₃ PO ₃ Et ₂ | | <0.0 04 | 2.3 | 29 | 176 | 824 | 171 | 233 | ND | ND | 195 |
| CO ₂ H | | 0.09 1 | 3.4 | 27 | 1548 | >600 0 | >600 0 | ND | ND | ND | ND |
| CONH(CH ₂) ₃ PO ₃ Et ₂ | | 0.15 7 | 1.6 | 10 | 188 | 476 | 666 | 240 | | | 319 |

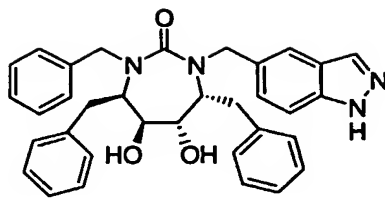


| Structure, R | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|--|------------------------|--------------------------------|--|---|------------|-------------------|-------------------|-------------------|-------------------|------------|
| | K _i (nM) | WT IC ₅₀ / nM | 84V9 0M IC ₅₀ / nM | WT | 84V9 0M | 30N 82I88 D | 48V5 4V82 A | 48V5 4V82 S | 48V8 2A90 M | 46I50 V |
| CH ₃ (DMP-851) | 0.033 | 3.8 | 9.4 | 54 | 918 | 69 | 33 | 30 | 22 | 17 |
| OH | 0.65 ^a | 6.1 | 77 | 356 | 2791 | 669 | 294 | ND | ND | 683 |
| OCH ₂ PO ₃ Et ₂ | 1.230 ^a | 23 | 157 | 356 | >600 0 | 145 | 175 | ND | ND | 138 |
| OCH ₂ PO ₃ H ₂ | 0.809 | 59 | 137 | 1074 | >600 0 | ND | ND | ND | ND | ND |
| O-mono-Lac-Et | >2.0 | 93 | 553 | >600 0 | >600 0 | ND | ND | ND | ND | ND |
| O-mono-Lac-Bu | >2.0 | 25 | 249 | >600 0 | >600 0 | ND | ND | ND | ND | ND |
| CH ₂ OH | 0.017 | 2.8 | 31 | 253 | 1106 | 486 | 413 | ND | ND | 524 |
| CH ₂ OCH ₂ PO ₃ Et ₂ | 2.8 | 13 | 123 | 119 | 3295 | 267 | 430 | ND | ND | 789 |
| CH ₂ OCH ₂ PO ₃ H ₂ | | 42 | 205 | 1757 | >424 3 | ND | ND | ND | ND | ND |

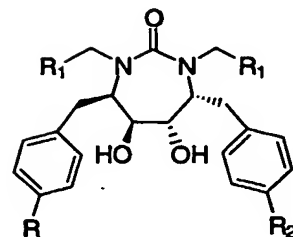
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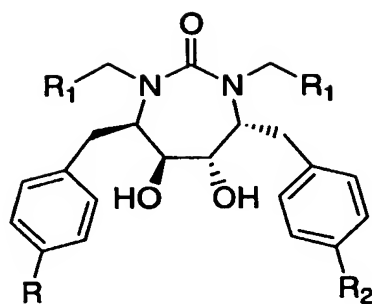
77546



277735

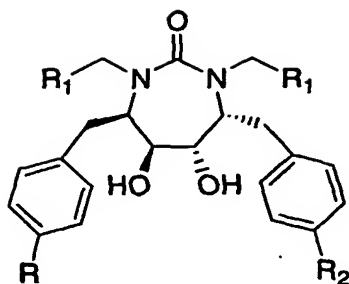


| | | | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|-----|---------|----------------------------------|------------------------|--------------------------------|--|---|------------|-------------------|-------------------|-------------------|-------------------|------------|
| R | R1 | R2 | K _i (nM) | WT IC ₅₀ / nM | 84V 90M IC ₅₀ / nM | WT | 84V 90M | 30N 82I8 8D | 48V 54V 82A | 48V 54V 82S | 48V 82A 90M | 46I5 0V |
| --- | --- | ---- | 0.03 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| --- | --- | --- | 0.37 | 5.8 | 43.3 | 193 | 2312 | 281 | 705 | ND | ND | 772 |
| H | Ph | H | | 34 | 631 | 2492 | >600 | 3360 | ND | ND | ND | ND |
| OH | Ph | OH | | 31 | 397 | 117 | 5609 | 756 | 2266 | ND | ND | 928 |
| OH | Ph | OCH ₂ PO ₃ | | 9 | 40 | 33 | 791 | 92 | 807 | 1103 | 1429 | 53 |
| H | Ph | OCH ₂ PO ₃ | 0.65 | 3.9 | 48 | 107 | 2456 | 293 | 1438 | 1899 | 3292 | 589 |
| H | Indazol | H | <0.0 | 2.5 | 13 | 11 | 22 | <8 | 5.5 | 8 | 4 | 4.0 |
| OH | Indazol | OH | 0.01 | 0.6 | 3.5 | >600 | 2728 | 7224 | ND | ND | ND | ND |
| OH | Indazol | OCH ₂ PO ₃ | 0.13 | 1.1 | 5.5 | 1698 | 1753 | 1998 | ND | ND | ND | ND |
| H | Indazol | OCH ₂ PO ₃ | 0.02 | 1.4 | 6.2 | 57 | 40 | 68 | 28 | 26 | 32 | 27 |



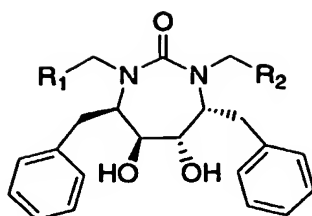
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| | | | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|------------------|------------------------------|---|---------------------|--------------------------|-------------------------------|---|---------|-------------|-------------|-------------|-------------|---------|
| R | R1 | R2 | K _i (nM) | WT IC ₅₀ / nM | 84V 90M IC ₅₀ / nM | WT | 84V 90M | 30N 82I8 8D | 48V 54V 82A | 48V 54V 82S | 48V 82A 90M | 46I5 0V |
| --- | ---- | ----- | 0.03 3 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| OH | Ph | OCH ₂ PO 3Et ₂ | | 9 | 40 | 33 | 791 | 92 | 807 | 1103 | 1429 | 53 |
| H | Ph | OCH ₂ PO 3Et ₂ | 0.65 6 | 3.9 | 48 | 107 | 2456 | 293 | 1438 | 1899 | 3292 | 589 |
| OCH ₃ | Ph | OCH ₂ PO 3Et ₂ | | | | | | | | | | |
| OH | Ph-pOH | OCH ₂ PO 3Et ₂ | <0.0 1 | 2.6 | 18 | 285 | 1912 | 211 | 986 | ND | ND | 1107 |
| H | Ph-pOH | OCH ₂ PO 3Et ₂ | 0.31 9 | 2.1 | 33 | 65 | 272 | 90 | 128 | 198 | 126 | 144 |
| OCH ₃ | Ph-pOH | OCH ₂ PO 3Et ₂ | 0.04 5 | 1.8 | 17 | 29 | 146 | 23 | 67 | 106 | 48 | 68 |
| OH | Ph-mNH ₂ /NHEt | OCH ₂ PO 3Et ₂ | | 8.7 | 67 | 286 | 1902 | 562 | 789 | 1781 | 684 | 239 |
| H | Ph-mNH ₂ | OCH ₂ PO 3Et ₂ | 0.12 6 | 3.4 | 39 | 65 | 328 | 16 | 168 | 146 | 74 | 46 |
| OCH ₃ | Ph-mNH ₂ | OCH ₂ PO 3Et ₂ | <0.0 1 | 3.6 | 56 | 63 | 535 | 18 | 202 | 117 | 102 | 36 |
| OCH ₃ | m- pyridine | OCH ₂ PO 3Et ₂ | | | | 115 | 765 | 106 | 1019 | 970 | 480 | 352 |



| | | | Enzymatic assay | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | | |
|----------------------|---------------------|--|------------------------|------------------------------|---|------|------------|-------------------|-------------------|-------------------|-------------------|------------|
| R | R1 | R2 | K _i (nM) | WT IC ₅₀ nM | 84 V9 0M IC ₅₀ nM | WT | 84V 90M | 30N 82I8 8D | 48V 54V 82A | 48V 54V 82S | 48V 82A 90M | 4615 0V |
| --- | --- | --- | 0.033 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| H | Ph-mNH ₂ | OCH ₂ PO ₃ Et ₂ | 0.126 | 3.4 | 39 | 65 | 328 | 16 | 168 | 146 | 74 | 46 |
| OC H ₃ | Ph-mNH ₂ | OCH ₂ PO ₃ Et ₂ | <0.01 | 3.6 | 56 | 63 | 535 | 18 | 202 | 117 | 102 | 36 |
| OC H ₃ | Ph-mNH ₂ | O(CH ₂) ₂ PO ₃ Et ₂ | | | | | | | | | | |
| OC H ₃ | Ph-mNH ₂ | OCONH (CH ₂) ₂ PO ₃ Et ₂ | | 11. 3 | 116 | 74 | 2265 | 77 | 262 | 214 | 215 | 184 |
| OC H ₃ | Ph-mNH ₂ | OCONH (CH ₂)PO ₃ Et ₂ | | 9.9 | 85 | 58 | 2151 | 68 | 223 | 203 | 185 | 104 |
| H | Ph-pOH | OCH ₂ PO ₃ Et ₂ | 0.319 | 2.1 | 33 | 65 | 272 | 90 | 128 | 222 | 146 | 144 |
| OC H ₃ | Ph-pOH | OCH ₂ PO ₃ Et ₂ | 0.045 | 1.8 | 17 | 30 | 148 | 25 | 70 | 129 | 54 | 90 |
| OC H ₃ | Ph-pOH | OCONH (CH ₂) ₂ PO ₃ Et ₂ | | 6.6 | 49 | 33 | 495 | 31 | 74 | 51 | 55 | 223 |
| --- | --- | --- | 0.033 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| H | Ph | OCH ₂ PO ₃ Et ₂ | 0.656 | 3.9 | 48 | 107 | 2456 | 293 | 1438 | 1899 | 3292 | 589 |
| H | Ph | OH | 0.330 | 15 | 162 | 1261 | >600 0 | 2952 | >600 0 | | | |
| H | Ph | OCH ₂ PO ₃ Bn ₂ | 0.125 | 7.4 | 158 | 1769 | >600 0 | 3135 | >600 0 | | | |
| H | Ph | OCH ₂ PO ₃ H ₂ | 0.386 | 9.7 | 210 | >600 | >600 | ND | ND | | | |

| | | | | | | | | | | | | |
|---|----|-------------|-------|-----|----|------|-----------|------|-----------|------|------|------|
| | | | | | | 0 | 0 | | | | | |
| H | Ph | Mono-lac-Et | 0.120 | 6.6 | 56 | 1726 | >600 0 | 2793 | >600 0 | | | |
| H | Ph | Mono-Ala-Et | | 5 | 50 | 310 | 2943 | 238 | 2851 | 1948 | 2450 | 1250 |



| R1 | R2 | Enzymatic assay | | | Cell-based assay (MT-4) EC ₅₀ / nM | | | | | | |
|--------|----|------------------------|--------------------------------|--|---|------------|-------------------|-------------------|-------------------|-------------------|------------|
| | | K _i (nM) | WT IC ₅₀ / nM | 84V 90 M IC ₅₀ / nM | WT | 84V9 0M | 30N 82I88 D | 48V5 4V82 A | 48V5 4V82 S | 48V8 2A90 M | 46I50 V |
| Phenyl | | 0.03 | 3.0 | 9.1 | 165 | 819 | 82 | 82 | 73 | 45 | 88 |
| Phenyl | | 0.42 | 6.6 | 85 | 1226 | >600 | 869 | 774 | ND | ND | 937 |
| Phenyl | | 0.37 | 5.8 | 43.3 | 193 | 2312 | 281 | 705 | ND | ND | 772 |
| Phenyl | | | 109 | >25 | >600 | ND | ND | ND | ND | ND | ND |
| Phenyl | | | | | | | | | | | |
| Phenyl | | | | | | | | | | | |
| Phenyl | | | | | | | | | | | |
| | | 1.43 | 302 | 114 | >600 | >600 | ND | ND | ND | ND | ND |
| | | >5 | >25 | ND | 5949 | ND | ND | ND | ND | ND | ND |
| | | >5 | 130 | 348 | 2006 | 3121 | ND | ND | ND | ND | ND |

5

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

10 Although certain embodiments have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

Example: Preliminary Study: Plasma and PBMC Exposure Following Intravenous and Oral Administration of Candidate to Beagle Dogs

5 The pharmacokinetics of a phosphonate prodrug GS77366 (P1-monoLac-iPr, structure shown below), its active metabolite (metabolite X, or GS77568), and GS8373 were studied in dogs following intravenous and oral administration of the candidate.

Dose Administration and Sample Collection. The in-life phase of this study was conducted in
10 accordance with the USDA Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and followed the standards for animal husbandry and care found in the Guide for the Care and Use of Laboratory Animals, 7th Edition, Revised 1996. All animal housing and study procedures involving live animals were carried out at a facility which had been accredited by the Association for Assessment and
15 Accreditation of Laboratory Animal Care - International (AAALAC).

Each animal in a group of 4 female beagle dogs was given a bolus dose of GS77366 (P1-monoLac-iPr) intravenously at 1 mg/kg in a formulation containing 40% PEG 300, 20% propylene glycol and 40% of 5% dextrose. Another group of 4 female beagle dogs was dosed
20 with GS77366 via oral gavage at 20 mg/kg in a formulation containing 60% Vitamin-E TPGS, 30% PEG 400 and 10% propylene glycol.

Blood samples were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-dose. Plasma (0.5 to 1 mL) was prepared from each sample and kept at -
25 70°C until analysis. Blood samples (8 mL) were also collected from each dog at 2, 8 and 24 hr post dose in Becton-Dickinson CPT vacutainer tubes. PBMCs were isolated from the blood by centrifugation for 15 minutes at 1500 to 1800 G. After centrifugation, the fraction containing PBMCs was transferred to a 15 mL conical centrifuge tube and the PBMCs were washed twice with phosphate buffered saline (PBS) without Ca²⁺ and Mg²⁺. The final wash of
30 the cell pellet was kept at -70°C until analysis.

Measurement of the candidate, metabolite X and GS8373 in plasma and PBMCs. For plasma sample analysis, the samples were processed by a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10 µM, from
35 J.T. Baker) were conditioned with 200 µL of methanol followed by 200 µL of water. An aliquot of 200 µL of plasma sample was applied to each cartridge, followed by two washing

steps each with 200 μ L of deionized water. The compounds were eluted from the cartridges with a two-step process each with 125 μ L of methanol. Each well was added 50 μ L of water and mixed. An aliquot of 25 μ L of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

5

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5 μ m) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, pH 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, pH 4.6. The chromatography was carried out at a flow rate of 250 μ L/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure GS77366, GS8373 and Metabolite X with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for GS77366, GS8373 and GS77568 (Metabolite X) in plasma.

10

For PBMC sample analysis, phosphate buffered saline (PBS) was added to each PBMC pellet to bring the total sample volume to 500 μ L in each sample. An aliquot of 150 μ L from each PBMC sample was mixed with an equal volume of methanol, followed by the addition of 700 μ L of 1% formic acid in water. The resulting mixture was applied to a Speedisk C18 solid phase extraction cartridge (1 mL, 20 mg, 10 μ m, from J.T. Baker) which had been conditioned as described above. The compounds were eluted with methanol after washing the cartridge 3 times with 10% methanol. The solvent was evaporated under a stream of N₂, and the sample was reconstituted in 150 μ L of 30% methanol. An aliquot of 75 μ L of the solution was injected for LC/MS/MS analysis. The limit of quantitation was 0.1 ng/mL in the PBMC suspension.

20

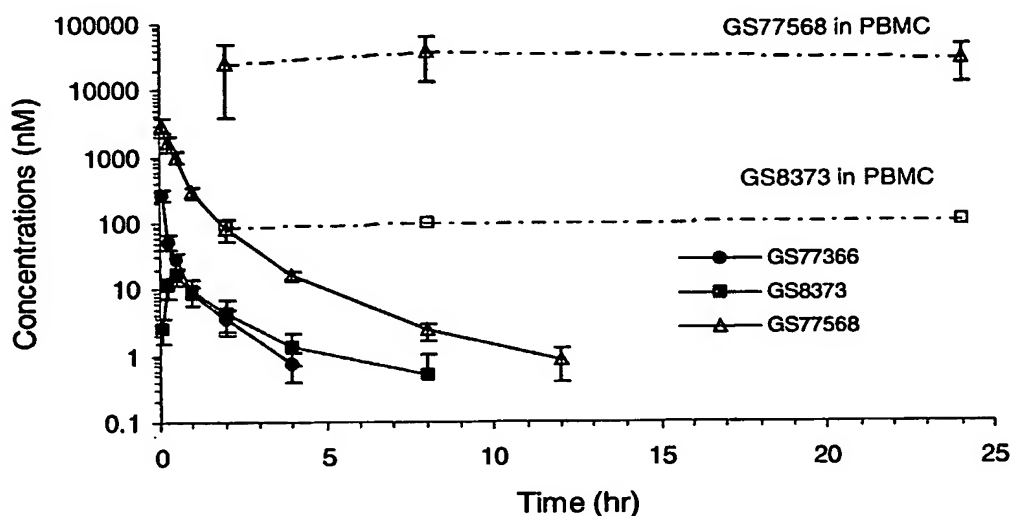
25

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were calculated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

30

Plasma and PBMC Concentration-time Profiles. The following shows the concentration-time profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous dosing of GS77366 at 1 mg/kg in dogs. The data demonstrate that the prodrug can effectively deliver the active components (metabolite X and GS8373) into cells that are primarily responsible for HIV replication, and that the active components in these cells had much longer half-life than in plasma.

Pharmacokinetic profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous administration of GS77366 at 1 mg/kg in dogs.



The pharmacokinetic properties of GS77568 in PBMCs following oral administration of GS77366 in dogs are compared with that of nelfinavir and amprenavir, two marketed HIV protease inhibitors. These data show that the active component (GS77568) from the phosphonate prodrug had sustained levels in PBMCs compared to nelfinavir and amprenavir.

Concentration-time profiles of GS77568, nelfinavir and amprenavir in PBMCs following oral administration of GS77366 (20 mg/kg), nelfinavir (17.5 mg/kg) and amprenavir (20 mg/kg) in dogs.

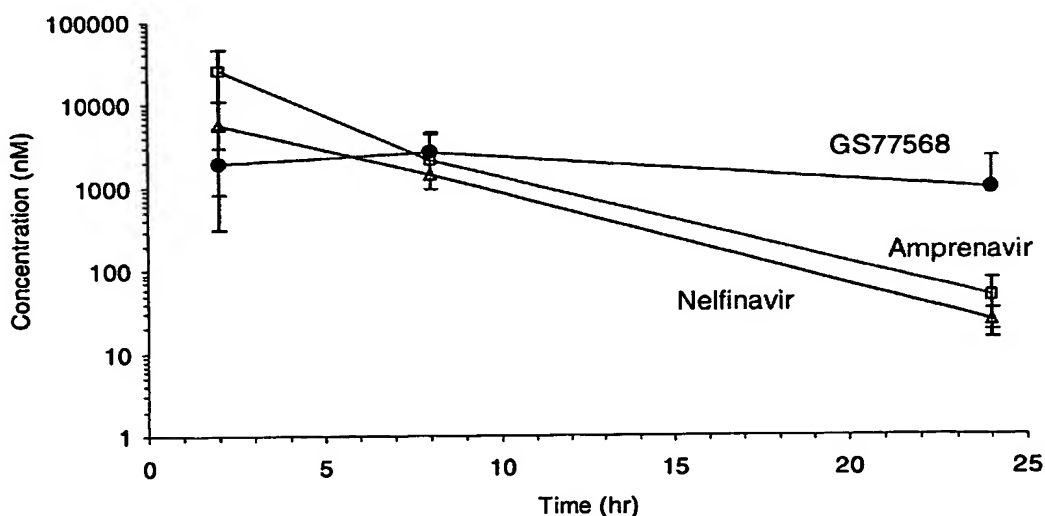


Table 1a. Comparison of GS77568 with nelfinavir and amprenavir in PBMCs following oral administration in beagle dogs.

| Compound | Dose | $t_{1/2}$ (hr) | AUC _(2-24 hr) |
|------------|---------------------|----------------|--------------------------|
| Nelfinavir | 17.5 mg/kg | 3.0 hr | 33,000 nM·hr |
| Amprenavir | 20 mg/kg | 1.7 hr | 102,000 nM·hr |
| GS77568 | 20 mg/kg of GS77366 | > 20 hr | 42,200 nM·hr |

5

Intracellular Metabolism/In Vitro Stability

1. Uptake and Persistence in MT2 cells, quiescent and stimulated PBMC

10 The protease inhibitor (PI) phosphonate prodrugs undergo rapid cell uptake and metabolism to produce acid metabolites including the parent phosphonic acid. Due to the presence of charges, the acid metabolites are significantly more persistent in the cells than non-charged PI's. In order to estimate the relative intracellular levels of the different PI prodrugs, three compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate, monoamidate phenoxy phosphonate and monolactate phenoxy phosphonate

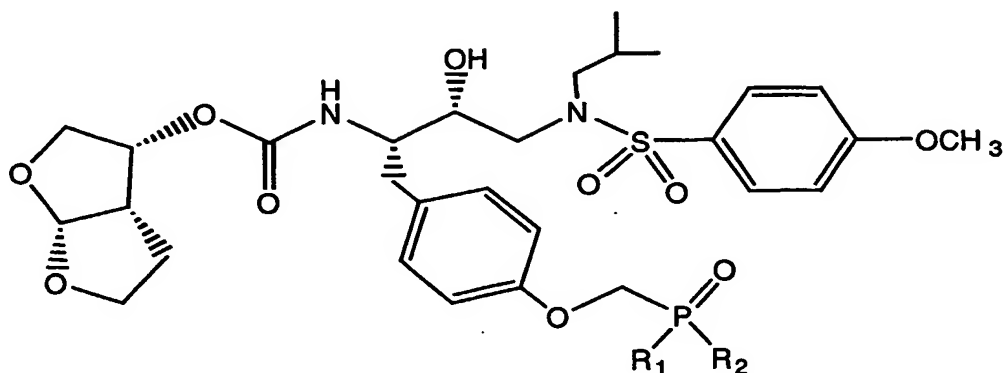
(Figure 1) were incubated at 10 μ M for 1 hr with MT-2 cells, stimulated and quiescent peripheral blood mononuclear cells (PBMC) (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 24 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. Typically, the cell lysates were centrifuged and 100 μ L of the supernatant were mixed with 200 μ L of 7.5 μ M amprenavir (Internal Standard) in 80% acetonitrile/20% water and injected into an HPLC system (70 μ L).

HPLC Conditions:

- 10 Analytical Column: Prodigy ODS-3, 75 x 4.6, 3 μ + C18 guard at 40°C
Gradient:
Mobile Phase A: 20 mM ammonium acetate in 10% ACN/90% H₂O
Mobile Phase B: 20 mM ammonium acetate in 70% ACN/30% H₂O
30-100%B in 4 min, 100%B for 2 min, 30%B for 2 min at 2.5 mL/min.
- 15 Run Time: 8 min
UV Detection @ 245 nm

Concentrations of Intracellular metabolites were calculated based on cell volume 0.2 μ L/mln cells for PBMC and 0.338 μ L / mln (0.676 μ L / mL) for MT-2 cells.

Chemical Structures of Selected Protease Inhibitor Phosphonate Prodrugs and Intracellular Metabolites.



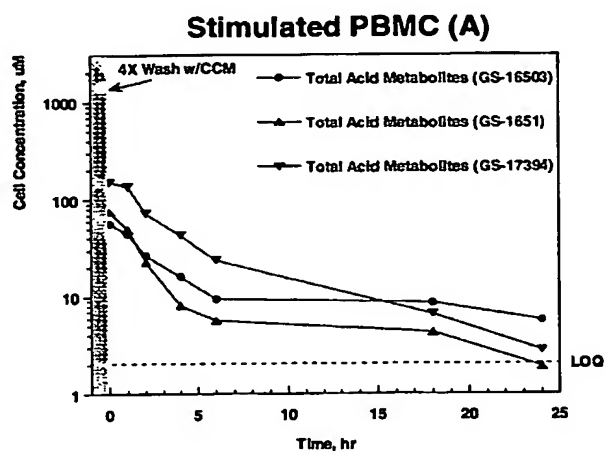
5

| GS No. | R1 | R2 | EC ₅₀ (nM) |
|--------|-----------------------------|---|-----------------------|
| 8373 | OH | OH | 4,800±1,800 |
| 16503 | HNCH(CH ₃)COOBu | HNCH(CH ₃)COOBu | 6.0±1.4 |
| 16571 | OPh | HNCH(CH ₃)COOEt | 15±5 |
| 17394 | OPh | OCH(CH ₃)COOEt | 20±7 |
| 16576 | OPh | HNCH(CH ₂ CH ₃)COOEt | 12.6±4.8 |
| Met X | OH | HNCH(CH ₃)COOH | >10,000 |
| Met LX | OH | OCH(CH ₃)COOEt | 1750±354 |

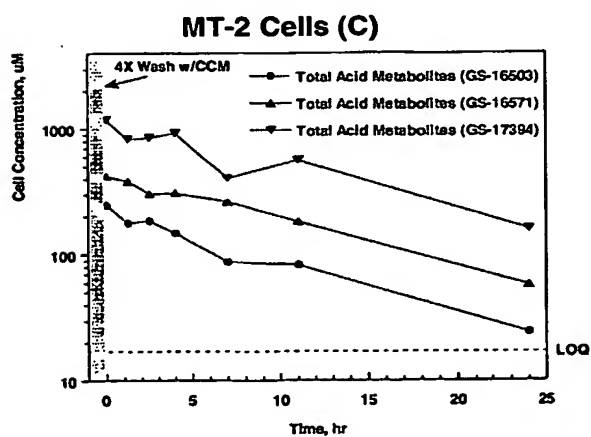
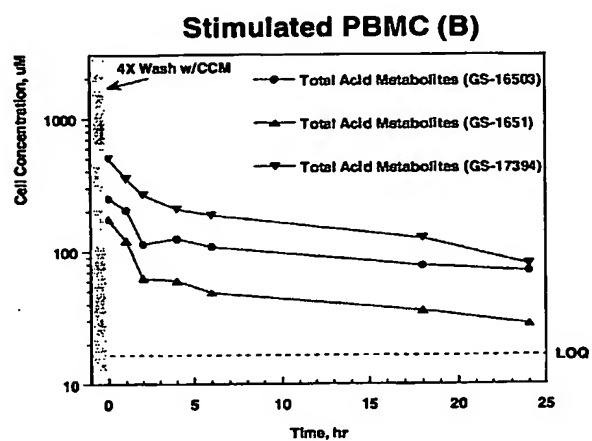
The foregoing data demonstrates that there was a significant uptake and conversion of all 3 compounds in all cell types. The uptake in the quiescent PBMC was 2-3-fold greater than in the stimulated cells. GS-16503 and GS-16571 were metabolized to Metabolite X and GS-8373. GS-17394 metabolized to the Metabolite LX. Apparent intracellular half-lives were similar for all metabolites in all cell types (7-12 hr).

10

Persistence of Total Acid Metabolites of Protease Inhibitor Prodrugs in Stimulated (A), Quiescent PBMC (B) and MT-2 Cells (C) (1 hr, 10 μ M Pulse, 24 hr Chase).



5



2. Uptake and Persistence in Stimulated and Quiescent T-cells

Since HIV mainly targets T-lymphocytes, it is important to establish the uptake, metabolism and persistence of the metabolites in the human T-cells. In order to estimate the relative intracellular levels of the different PI prodrugs, GS-16503, 16571 and 17394 were incubated at 10 μ M for 1 hr with quiescent and stimulated T-cells (pulse phase). The prodrugs were compared with a non-prodrug PI, nelfinavir. After incubation, the cells were washed, resuspended in the cell culture media and incubated for 4 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC.

Table 1b demonstrates the levels of total acid metabolites and corresponding prodrugs in T-cells following pulse/chase and continuous incubation. There was significant cell uptake/metabolism in T-lymphocytes. There was no apparent difference in uptake between stimulated and quiescent T-lymphocytes. There was significantly higher uptake of phosphonate PI's than nelfinavir. GS17394 demonstrates higher intracellular levels than GS16571 and GS16503. The degree of conversion to acid metabolites varied between different prodrugs. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equal mixture of the mono-phosphonic acid metabolite and GS-8373 except for GS-17394, where Metabolite LX was stable, with no GS-8373 formed.

Table 1b. Intracellular Levels of Metabolites and Intact Prodrug Following Continuous and 1 hr Pulse/4 hr Chase Incubation (10 μ M/0.7 mln cells/1 mL) of 10 μ M PI Prodrugs and Nelfinavir with Quiescent and Stimulated T-cells

| Compound | Time (h) | Continuous Incubation | | | | 1 hr Pulse /4 hr Chase | | | |
|------------|----------|-----------------------|--------------------|---------------------|--------------------|------------------------|--------------------|---------------------|--------------------|
| | | Quiescent T-cells | | Stimulated T-cells | | Quiescent T-cells | | Stimulated T-cells | |
| | | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) | Acid Met (μ M) | Prodrug (μ M) |
| 16503 | 0 | 1180 | 42 | 2278 | 0 | 2989 | 40 | 1323 | 139 |
| | 2 | 3170 | 88 | 1083 | 116 | 1867 | 4 | 1137 | 31 |
| | 4 | 5262 | 0 | 3198 | 31 | 1054 | 119 | 1008 | 0 |
| 16571 | 0 | 388 | 1392 | 187 | 1417 | 1042 | 181 | 858 | 218 |
| | 2 | 947 | 841 | 1895 | 807 | 1170 | 82 | 1006 | 35 |
| | 4 | 3518 | 464 | 6147 | 474 | 1176 | 37 | 616 | 25 |
| 17394 | 0 | 948 | 1155 | 186 | 1194 | 4480 | 14 | 2818 | 10 |
| | 2 | 7231 | 413 | 3748 | 471 | 2898 | 33 | 1083 | 51 |
| | 4 | 10153 | 167 | 3867 | 228 | 1548 | 39 | 943 | 104 |
| Nelfinavir | 0 | | 101 | | 86 | | 886 | | 1239 |
| | 2 | | 856 | | 846 | | 725 | | 770 |
| | 4 | | 992 | | 1526 | | 171 | | 544 |

5

3. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μ M.

To determine if the cell uptake/metabolism is concentration dependent, selected PI's were incubated with the 1 mL of MT-2 cell suspension (2.74 mln cells/mL) for 1 hr at 37°C at 3

- 10 different concentrations: 10, 5 and 1 μ M. Following incubation, cells were washed twice with the cell culture medium, lysed and assayed using HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC. Intracellular concentrations were calculated based on cell count, a published single cell volume of 0.338 pl for MT-2 cells, and concentrations of analytes in cell
- 15 lysates. Data are shown in Table 2a.

- Uptake of all three selected PI's in MT-2 cells appears to be concentration-independent in the 1-10 μ M range. Metabolism (conversion to acid metabolites) appeared to be concentration-dependent for GS-16503 and GS-16577 (3-fold increase at 1 μ M vs. 10 μ M) but independent for GS-17394 (monolactate). Conversion from a respective metabolite X to GS-8373 was
- 20 concentration-independent for both GS-16503 and GS-16577 (no conversion was observed for metabolite LX of GS-17394).

Table 2a. Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μ M.

| Compound | Extracellular Concentration, μ M | Cell-Associated Prodrug and Metabolites Concentration, μ M | | | | % Conversion to acid metabolites |
|----------|--------------------------------------|--|--------|---------|-------|----------------------------------|
| | | Metabolite X | GS8373 | Prodrug | Total | |
| GS-17394 | 10 | 1358 | 0 | 635 | 1993 | 68 |
| | 5 | 916 | 0 | 449 | 1365 | 67 |
| | 1 | 196 | 0 | 63 | 260 | 76 |
| GS-16576 | 10 | 478 | 238 | 2519 | 3235 | 22 |
| | 5 | 250 | 148 | 621 | 1043 | 40 |
| | 1 | 65 | 36 | 61 | 168 | 64 |
| GS-16503 | 10 | 120 | 86 | 1506 | 1712 | 12 |
| | 5 | 58 | 60 | 579 | 697 | 17 |
| | 1 | 12 | 18 | 74 | 104 | 29 |

5 * For GS16576, Metabolite X is mono-aminobutyric acid

4. PBMC Uptake and Metabolism of Selected PI Candidates Following 1-hr Incubation in Human Whole Blood at 10 μ M.

In order to estimate the relative intracellular levels of the different PI prodrugs candidates under conditions simulating the in vivo environment, compounds representative of three

5 classes of phosphonate PI prodrugs – bisamidate phosphonate (GS-16503), monoamidate phenoxy phosphonate (GS-16571) and monolactate phenoxy phosphonate (GS-17394) (Figure 1) were incubated at 10 μ M for 1 hr with intact human whole blood at 37°C. After incubation, PBMC were isolated, then lysed and the lysates were analyzed by HPLC with UV detection. The results of analysis are shown in Table 3. There was significant cell uptake/metabolism
10 following incubation in whole blood. There was no apparent difference in uptake between GS-16503 and GS-16571. GS-17394 demonstrated significantly higher intracellular levels than GS-16571 and GS-16503.

The degree of conversion to acid metabolites varies between different prodrugs after 1 hr
15 incubation. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equimolar mixture of the mono-phosphonic acid metabolite and GS-8373 (parent acid) except for GS-17394, where Metabolite LX was stable with no GS-8373 formed.

Table 3a. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10 μ M (Mean \pm SD, N=3).

| GS# | Intracellular Prodrug and Metabolites Concentration, μ M | | | Major Intracellular Metabolites |
|-------|--|------------------|----------------|---------------------------------|
| | Acid Metabolite | Prodrug, μ M | Total, μ M | |
| 16503 | 279 \pm 47 | 61 \pm 40 | 340 \pm 35 | X, GS-8373 |
| 16571 | 319 \pm 112 | 137 \pm 62 | 432 \pm 208 | X, GS-8373 |
| 17394 | 629 \pm 303 | 69 \pm 85 | 698 \pm 301 | LX |

5 * PBMC Intracellular Volume = 0.2 μ L/mln

5. Distribution of PI Prodrug Candidates in PBMC

In order to compare distribution and persistence of PI phosphonate prodrugs with those of non-prodrug PI's, GS-16503, GS-17394 and nelfinavir, were incubated at 10 μ M for 1 hr with PBMC (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 20 more hr (chase phase). At specific time points, the cells were washed and lysed. The cell cytosol was separated from membranes by centrifugation at 9000 x g. Both cytosol and membranes were extracted with acetonitrile and analyzed by HPLC with UV detection.

Table 4a and the accompanying bar graphs below show the levels of total acid metabolites and corresponding prodrugs in the cytosol and membranes before and after the 22 hr chase. Both prodrugs exhibited complete conversion to the acid metabolites (GS-8373 and X for GS-16503 and LX for GS-17394, respectively). The levels of the acid metabolites of the PI phosphonate prodrugs in the cytosol fraction were 2-3-fold greater than those in the membrane fraction after the 1 hr pulse and 10-fold greater after the 22 hr chase. Nelfinavir was present only in the membrane fractions. The uptake of GS-17394 was about 3-fold greater than that of GS-16503 and 30-fold greater than nelfinavir.

The metabolites were an equimolar mixture of metabolite X and GS-8373 (parent acid) for GS-16503 and only metabolite LX for GS-17394.

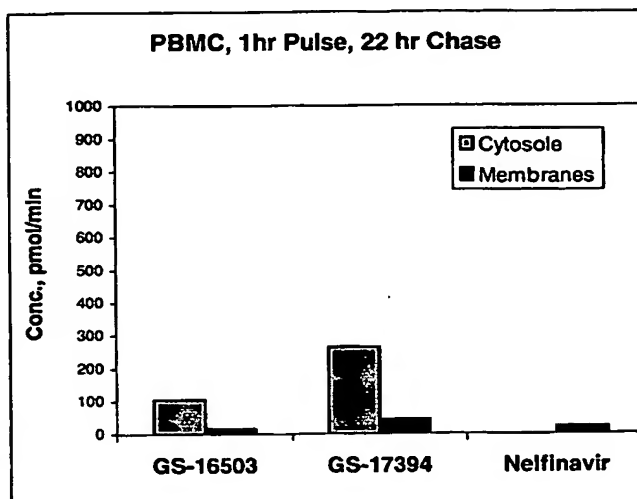
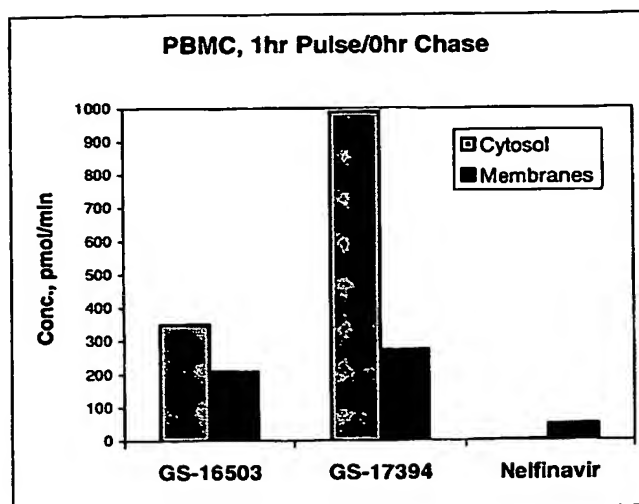
Table 4a. Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following Continuous and 1 hr Pulse/22 hr Chase Incubation of 10 uM PI Prodrugs and Nelfinavir with Quiescent PBMC.

5

| GS# | Cell Type | Fraction | Cell-Associated PI, pmol/mln cells | | | |
|------------|-----------|----------|------------------------------------|---------|-------------------------|---------|
| | | | 1 hr Pulse/ 0 hr Chase | | 1 hr Pulse/ 22 hr Chase | |
| | | | Acid Metabolites | Prodrug | Acid Metabolites | Prodrug |
| GS-16503 | PBMC | Membrane | 228 | 0 | 9 | 0 |
| GS-16503 | PBMC | Cytosol | 390 | 0 | 130 | 0 |
| GS-17394 | PBMC | Membrane | 335 | 0 | 26 | 0 |
| GS-17394 | PBMC | Cytosol | 894 | 0 | 249 | 0 |
| Nelfinavir | PBMC | Membrane | | 42 | | 25 |
| Nelfinavir | PBMC | Cytosol | | 0 | | 0 |

Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following 1 hr Pulse/22 hr Chase Incubation of 10 uM PI Prodrugs and Nelfinavir with Quiescent PBMC.

5



6. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrug Candidates

The in vitro metabolism and stability of the PI phosphonate prodrugs were determined in PBMC extract, dog plasma and human serum. Biological samples listed below (120 μ L) were transferred into an 8-tube strip placed in the aluminum 37°C heating block/holder and incubated at 37°C for 5 min. Aliquots (2.5 μ L) of solution containing 1 mM of test compounds in DMSO, were transferred to a clean 8-tube strip, placed in the aluminum 37°C heating block/holder. 60 μ L aliquots of 80% acetonitrile/20% water containing 7.5 μ M of amprenavir as an internal standard for HPLC analysis were placed into five 8-tube strips and kept on ice/refrigerated prior to use. An enzymatic reaction was started by adding 120 μ L aliquots of a biological sample to the strip with the test compounds using a multichannel pipet. The strip was immediately vortex-mixed and the reaction mixture (20 μ L) was sampled and transferred to the Internal Standard/ACN strip. The sample was considered the time-zero sample (actual time was 1-2 min). Then, at specific time points, the reaction mixture (20 μ L) was sampled and transferred to the corresponding IS/ACN strip. Typical sampling times were 6, 20, 60 and 120 min. When all time points were sampled, an 80 μ L aliquot of water was added to each tube and strips were centrifuged for 30 min at 3000xG. The supernatants were analyzed with HPLC under the following conditions:

Column: Inertsil ODS-3, 75 x 4.6 mm, 3 μ m at 40°C.

Mobile Phase A: 20 mM ammonium acetate in 10%ACN/90%water

Mobile Phase B 20 mM ammonium acetate in 70%ACN/30%water

Gradient: 20% B to 100% B in 4 min, 2 min 100% B, 2 min 20% B

Flow Rate: 2 mL/min

Detection: UV at 243 nm

Run Time: 8 min

The biological samples evaluated were as follows:

PBMC cell extract was prepared from fresh cells using a modified published procedure (A.

Pompon, I. Lefebvre, J.-L. Imbach, S. Kahn, and D. Farquhar, Antiviral Chemistry &

Chemotherapy, 5, 91 - 98 (1994)). Briefly, the extract was prepared as following: The cells were separated from their culture medium by centrifugation (1000 g, 15 min, ambient

temperature). The residue (about 100 μ L, 3.5×10^8 cells) was resuspended in 4 mL of a

buffer (0.010 M HEPES, pH 7.4, 50 mM potassium chloride, 5 mM magnesium chloride and 5

mM dl-dithiothreitol) and sonicated. The lysate was centrifuged (9000 g, 10 min, 4°C) to remove membranes. The upper layer (0.5 mg protein/mL) was stored at -70°C. The reaction mixture contained the cell extract at about 0.5 mg protein/mL.

Human serum (pooled normal human serum from George King Biomedical Systems, Inc.).

5 Protein concentration in the reaction mixture was about 60 mg protein/mL.

Dog Plasma (pooled normal dog plasma (EDTA) from Pel Freez, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

Table 5a. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

| GS# | PBMC Extract ¹ T _{1/2} , min | Dog Plasma T _{1/2} , min | Human Serum T _{1/2} , min | HIV EC ₅₀ (nM) |
|-------|--|---|--|------------------------------|
| 16503 | 2 | 368 | >>400 | 6.0 ± 1.4 |
| 16571 | 49 | 126 | 110 | 15 ± 5 |
| 17394 | 15 | 144 | 49 | 20 ± 7 |

Example: Pharmacokinetics in Plasma and PBMC Following Intravenous or Oral Administration of Candidate compounds to Beagle Dogs; Method for Determining Intracellular Residence Time

15 The pharmacokinetics of several candidate compounds and their active metabolites were studied in beagle dogs following intravenous or oral administration of each candidate compound.

20 **Dose Administration and Sample Collection.** Each dosing group consisted of 3 male beagle dogs that were fasted overnight before dosing. For intravenous administration, each dog was dosed with the candidate compound at 1 mg/kg via the cephalic vein as a slow bolus injection over approximately 1 minute. Blood samples (1-2 mL) were collected from the jugular vein pre-dose, and at 2 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr post-dose
25 into tubes containing EDTA as the anticoagulant. For oral administration, each dog was dosed with the candidate compound at 4 mg/kg through oral gavage. Blood samples (1-2 mL) were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 12 hr post-dose into tubes containing EDTA as the anticoagulant. The blood samples were stored on ice

and plasma samples were obtained by centrifugation within 1 hour after blood collection. The plasma samples were stored at approximately -70°C until analysis for the concentrations of the candidate compound and its metabolites in plasma.

5 Another set of blood samples was also collected from the jugular vein for evaluation of the concentrations of candidate compound and its metabolites in peripheral blood mononuclear cells (PBMCs). Approximately 8 mL of blood was collected either at 1 hr, 4 hr, 8 hr and 24 hr post-dose or at 2 hr, 8 hr and 24 hr post-dose from the jugular vein into tubes containing EDTA as the anticoagulant. An equal volume of sterile phosphate buffered saline (PBS) was
10 mixed with each blood sample. The mixture was laid over 15 mL of Ficoll-Paque (Amersham Biosciences) in a 50 mL conical tube. The tube was centrifuged at approximately 500 g for 30 min at room temperature. The upper layer containing plasma was drawn off and discarded. The layer below the plasma layer is enriched with PBMCs. This layer was collected with a clean pipette and transferred to a 15 mL conical tube. The PBMC suspension was centrifuged
15 at approximately 500 g for 10 min at room temperature. The resulting pellet was resuspended in 5 mL of sterile PBS and then centrifuged at approximately 500 g for 10 min at room temperature. The supernatant was removed and 0.5 mL of acetonitrile was added to the pellet. The tube was vortexed, sealed and stored at -70°C until analysis for concentrations of the candidate compound and its metabolites.

20 **Determination of the concentrations of the candidate compound and its metabolites in plasma.** The plasma concentrations of the candidate compound and its metabolites were determined by an LC/MS/MS assay. The plasma samples were processed with a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1
25 mL, 20 mg, 10 µm, from J.T. Baker) in a 96-well plate were conditioned with 200 µL of methanol followed by 200 µL of water. An aliquot of 200 µL of plasma sample was applied to each cartridge, followed by two washing steps each with 200 µL of deionized water. The analytes were eluted from the cartridges by a two-step process each with 125 µL of methanol. Each well was added 50 µL of water and mixed to reduce the organic strength. An aliquot of
30 25 µL of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography (LC) was HyPURITY® C18 (50 x 2.1 mm, 3.5 µm) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. Mobile phase B contained 90% acetonitrile in 10 mM ammonium

formate, 0.1% formic acid. The chromatography was carried out at a flow rate of 250 μ L/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure the candidate compound and its metabolites simultaneously with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for the candidate compound and its metabolites in plasma.

Determination of the concentrations of the candidate compound and its metabolites in PBMCs.

The concentrations of the candidate compound and its metabolites in PBMCs were determined by an LC/MS/MS assay. The PBMC samples were filtered through a Captiva™ filtration plate with 0.2 μ m pore size. An aliquot of 250 μ L of the filtrate was evaporated under a stream of nitrogen. The samples were reconstituted in 75 μ L of 20% acetonitrile in 0.1% formic acid. An aliquot of 25 μ L of the solution was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5 μ m) from Thermo-Hypersil. Mobile phase A (MPA) contained 10% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. Mobile phase B (MPB) contained 90% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. The chromatography was carried out at a flow rate of 300 μ L/min with a gradient elution program: 5% MPB from 0 to 1.5 min; 5-95% MPB from 1.5 to 1.6 min; 95% MPB from 1.6 to 3.5 min; 95-5% MPB from 3.5 to 3.6 min; 5% MPB till the end of the program (6 min). The first 2 min of the LC flow was diverted to waste to alleviate salt buildup in the probe of the mass spectrometer. Selected reaction monitoring was used to measure the candidate compound and its metabolites simultaneously with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 0.1 nM for the candidate compound and its metabolites in PBMC suspension.

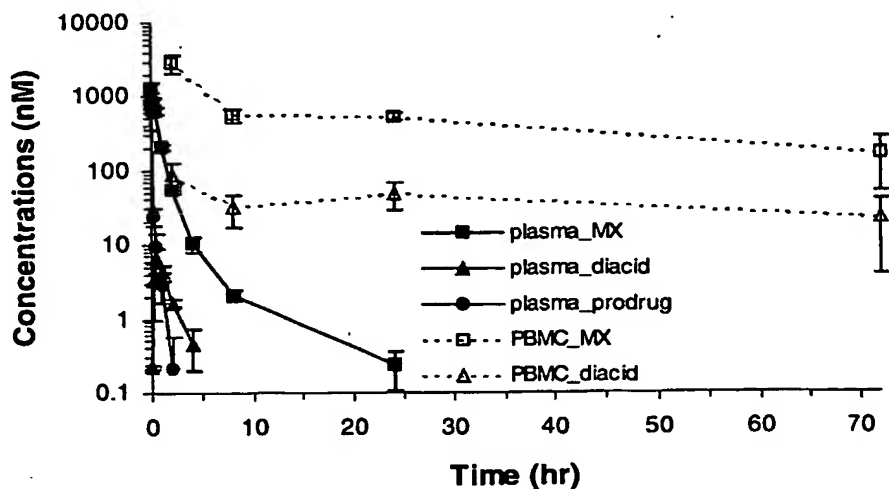
Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were extrapolated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

Pharmacokinetic Profiles in Plasma and PBMC. Shown below are the concentration-time profiles of three phosphonate candidate compounds (GS-1, GS-2 and GS-3) and their metabolites in plasma and PBMCs following intravenous administration of each candidate compound at 1 mg/kg in dogs. The last profile shows the concentration-time profiles of GS-3 and its metabolites in plasma and PBMC following oral administration of GS-3 at 4 mg/kg in dogs. The chemical structures of the candidate compounds and their metabolites are shown in Table 1aa. The data demonstrate that the candidate compounds can effectively deliver the active components (metabolite X and diacid) into cells that are primarily associated with HIV activity, and that the half-lives of the active components in these cells are much longer than in plasma.

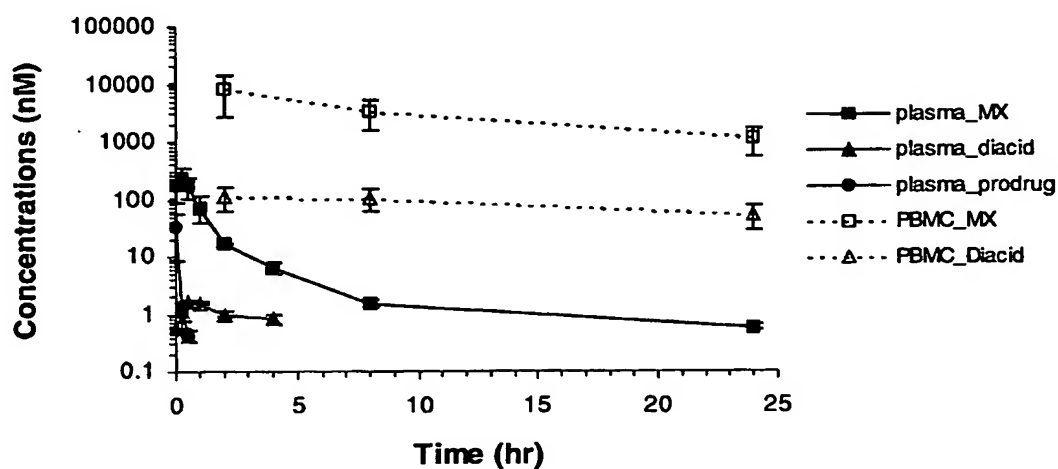
Table 1aa. Chemical Structures of Candidate compounds and Their Metabolites.

| | Candidate compound | Metabolites | |
|------|--------------------|-------------------|--------|
| | | Metabolite X (MX) | Diacid |
| GS-1 | | | |
| GS-2 | | | |
| GS-3 | | | |

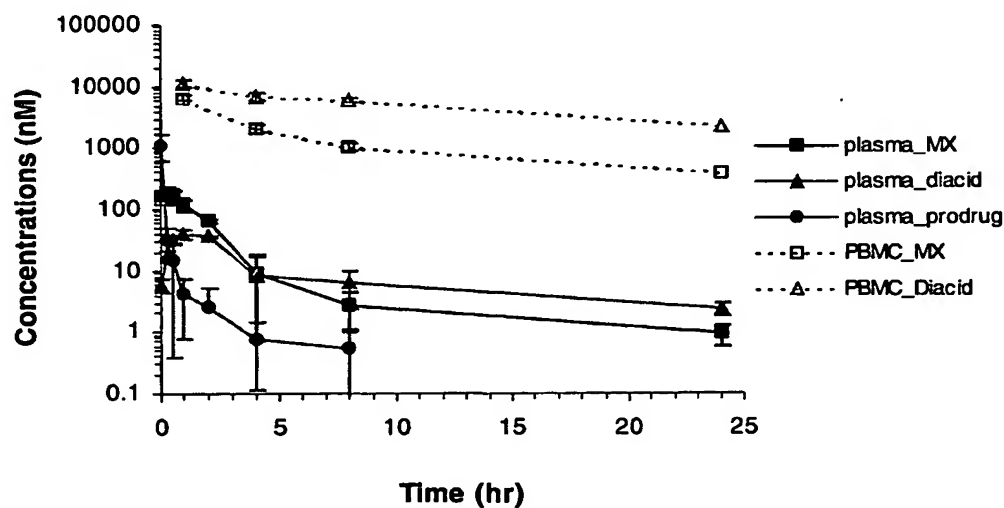
Pharmacokinetic profiles of GS-1 and its metabolites in plasma and PBMCs following intravenous administration of GS-1 at 1 mg/kg in dogs.



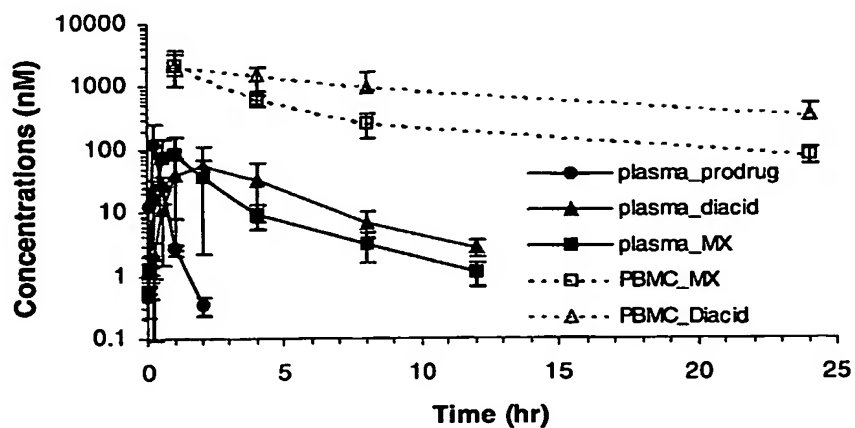
Pharmacokinetic profiles of GS-2 and its metabolites in plasma and PBMCs following intravenous administration of GS-2 at 1 mg/kg in dogs.



5 Pharmacokinetic profiles of GS-3 and its metabolites in plasma and PBMCs following intravenous administration of GS-3 at 1 mg/kg in dogs.



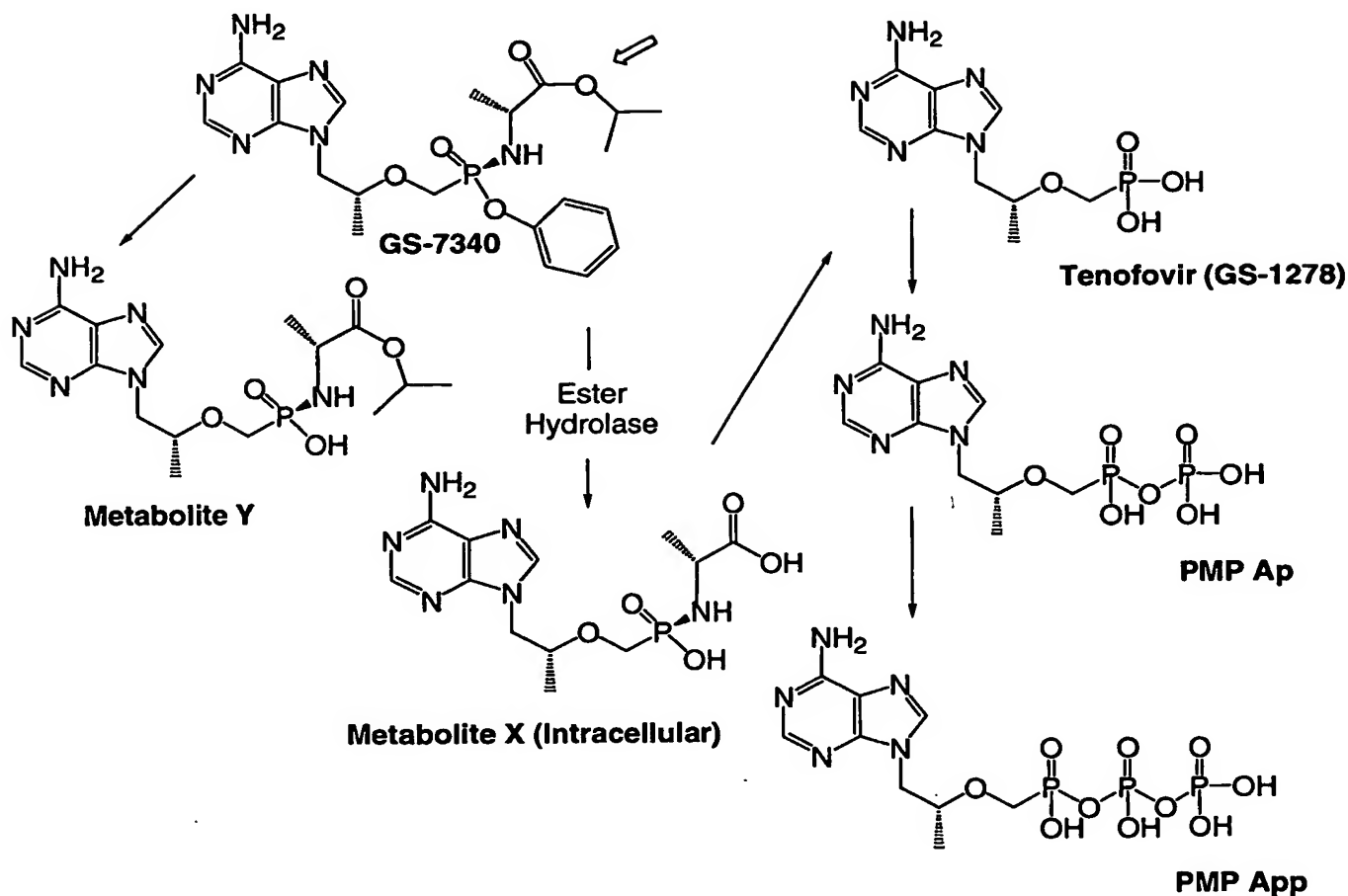
Pharmacokinetic profiles of GS-3 and its metabolites in plasma and PBMCs following oral administration of GS-3 at 4 mg/kg in dogs.



5

Example: Purification and Biochemical Characterization of GS-7340 Ester Hydrolase:

Major Metabolites of GS-7340



Metabolism of GS-7340:

There is broad consensus that the bioactivation of nucleotide amidate triesters follows a general scheme (Figure 1) (Valette, 1996; McGuigan, 1998a, 1998b; Saboulard, 1999; Siddiqui, 1999). Step A is the hydrolysis of the amino acid carboxylic ester. A nucleophilic attack by the carboxylic acid of the phosphorous (StepB) is believed to initiate the formation of the 5-membered cyclic intermediate which in turn is quickly hydrolyzed to the monoamidate diester (referred to as the amino acid nucleoside monophosphate, AAM, or metabolite X, Step C). This compound is considered an intracellular depot form of the antiviral nucleoside. Various enzymes as well as non-enzymatic catalysis have been implicated in Step D which is the hydrolysis of the amide bond resulting in the formation of the nucleotide. The nucleotide is activated by enzymatic phosphorylation to nucleotide di- and tri-phosphates.

In the case of GS-7340, the efficient conversion of this pro-drug to the amino acid nucleoside monophosphate (Metabolite X, Figure 2) is a necessary step for the observed accumulation of Metabolite X in peripheral blood mononuclear cells (PBMC). Purification of the Enzyme(s) responsible for the cleavage of GS-7340 amino acid carboxylic ester resulting in the formation of Metabolite X is the subject of this example.

Ester Hydrolase Assay:

The enzymatic production of metabolite X from GS-7340 was monitored using the following Ester Hydrolase assay: Varying amounts of peripheral blood mononuclear cell (PBMC) extracts, column fractions or pools were incubated with [^{14}C] GS-7340 at 37°C for 10 – 90 min. The production of [^{14}C] Metabolite X was monitored by measuring the amount of radioactivity retained on an anion exchange resin (DE-81). HPLC and mass spectrometry analysis of the reaction mixture and radioactivity retained on the filter confirmed that only [^{14}C]-Metabolite X bound the DE-81 filter. Under the assay conditions, the more hydrophobic [^{14}C] GS-7340 is not retained on the DE-81 membrane. The final reaction conditions were: 25 mM 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.5, 100mM NaCl, 1 mM DTT, 30 μM [^{14}C] GS-7340, 0.1% NP40 and varying amounts of enzyme in a final volume of 60 μl . The reaction mixture was incubated at 37°C and at 10, 30 and 90 minutes, 17 μl of the reaction mixture was spotted onto a DE-81 filter. The filter was washed with 25mM Tris, pH 7.5 100mM NaCl, dried at room temperature, placed in vials containing 5ml of scintillation fluid. [^{14}C]-Metabolite X present on the filters was determined using a scintillation counter (LS 6500, Beckman, _____). Activity was expressed as pmoles Metabolite X produced / minute / volume enzyme sample. Ester Hydrolase Specific Activity was expressed as pmoles Metabolite X produced / minute / μg protein.

Non-Specific Esterase Assay:

Non-specific ester hydrolase activity was monitored by monitoring the enzymatic cleavage of alpha naphthyl acetate (ANA) (Mastropaolo, W and Yourno, J 1981). This substrate has been used for both the measurement esterase enzyme activity and *in situ* staining of esterases in tissue samples (Yourno, J and Mastropaolo, W. 1981; Yourno, J et Al 1981; Yourno, J et al 1986). The method described is a modification of the assay described by Mattes, P and Mattes, WB, 1992). Varying amounts of peripheral blood mononuclear cell (PBMC) extracts column fractions or pools were incubated with ANA at 37°C for 20 min. The final reaction

conditions were: 10 mM sodium phosphate, pH 6.5, 97 μ M ANA and varying amounts of enzyme in a final volume of 150 μ l. The reaction mixture was incubated at 37°C and at 20 minutes, and the reaction was stopped by the addition of 20 μ l of 10mM Blue salt RR in 10% sodium dodecyl sulfate (SDS). The alpha naphthyl-Blue salt RR product was detected by
 5 reading absorbance at 405nm. Activity was expressed as pmoles product produced /minute/ volume enzyme sample.

Extraction of GS-7340 Ester Hydrolase from Human PBMCs:

10 Fresh human PBMC were obtained from patients undergoing leukapheresis; cells were shipped in plasma and processed within 26 h of draw. PBMC cells were harvested by centrifugation at 1200 X g for 5minutes and washed three times by re-suspension in RBC lysis buffer (155 mM NH_4Cl , 1 mM EDTA, 10mM KHCO_3). Washed cells (29×10^9) were
 15 suspended in 150 ml of lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 20 mM CaCl_2 , 1 mM DTT and 1% NP40) and incubated on ice for 20 minutes. The PBMC crude extract was centrifuged at 1000 X g for 30 min to remove unlysed cells and the supernatant at 100,000 X g for 1h. The 100,000 X g supernatant (PBMC Extract: P0) was harvested (165ml) and the pellets (1000 X g and 100,000 X g pellets) were resuspended in 10 mM Tris, pH 7.4, 150 mM NaCl, 20 mM CaCl_2 , 1 mM DTT and assayed for GS-GS-7340 ester hydrolase activity.
 20 Assays showed that < 2% of the GS-GS-7340 Ester Hydrolase enzymatic activity was present in the pellets. The cell extract was snap frozen in liquid Nitrogen and stored at -70°C.

Anion Exchange Chromatography:

25 The PBMC Extract (15×10^9 cells, 75 – 85ml) was diluted 1:10, (vol: vol) with 25mM Tris, pH 7.5, 10% glycerol, 1mM DTT (Q15 Buffer A) and loaded onto an anion exchange column (2.5cm X 8.0 cm, Source Q15 (Amersham Biosciences)), previously equilibrated with Q15 Buffer A. Bound protein was eluted with a linear NaCl gradient (30 column volumes (CV)) to 0.5M NaCl. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions
 30 (12.0 ml) were collected and assayed for both GS-7340 Ester Hydrolase and ANA Esterase activity. GS-7340 Ester Hydrolase activity eluted as a single major peak at 50 – 75 mM NaCl (Table 1). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 50 – 65% of total activity loaded. Significant ANA Esterase activity (30-40% of total activity loaded) was detected in the column FT; however, ~ 30% eluted in two peaks at 70 – 100 mM

NaCl (Table 1). Fractions containing GS-7340 Ester Hydrolase activity (Q15 pool) were pooled, snap frozen in liquid nitrogen and stored at -70°C .

Hydrophobic Interaction (HIC) Chromatography:

5 The Q15 pool was defrosted and diluted 1:1, (vol: vol) with 25mM Tris, pH 8.0, 0.5 M $(\text{NH}_4)_2\text{SO}_4$, 1mM DTT, 10% glycerol BS-HIC Buffer A). 1M $(\text{NH}_4)_2\text{SO}_4$ was added to yield a final concentration of 0.5M $(\text{NH}_4)_2\text{SO}_4$ in the sample. The sample (300ml / 10×10^9 cells) was loaded onto a Butyl Sepharose HIC column (5ml HiTrap, Amersham Biosciences) previously equilibrated with BS-HIC Buffer A. Bound protein was eluted with a linear
10 gradient (15 CV) decreasing to with 25mM Tris, pH 8.0, 1mM DTT, 10% glycerol. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (4.0 ml) were collected and assayed for both GS-7340 Ester Hydrolase and ANA Esterase activity. GS-7340 Ester Hydrolase activity eluted as a single major peak at 200 – 75 mM $(\text{NH}_4)_2\text{SO}_4$ (Table 1). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 50 – 65% of
15 total activity loaded (Table 1). Significant ANA Esterase activity (85% of total activity loaded) was detected in the column FT; however, ~ 10-15% eluted in a peak at 450 – 300 mM $(\text{NH}_4)_2\text{SO}_4$. Fractions containing GS-7340 Ester Hydrolase activity (BS-HIC pool) were pooled, snap frozen in liquid nitrogen and stored at -70°C .

Hydroxyapatite (HAP) Chromatography:

20 The BS-HIC pool (40 ml / 10×10^9 cells) was defrosted, concentrated to 2.0ml using a 10kDa molecular weight cutoff concentrator (20ml Vivaspin concentrator, Viva Science, Carlsbad, CA), and diluted to 20ml with 1mM sodium phosphate, pH 6.85, 10% glycerol, 1mM DTT (HAP Buffer A). The sample containing the GS-7340 Ester Hydrolase activity was loaded
25 onto a HAP column (0.75 ml, 5mm X 20mm; ceramic hydroxyapatite, BioRad, Hercules, CA), previously equilibrated with HAP Buffer A. Bound protein was eluted with a 40 CV gradient to 500 mM sodium phosphate, pH 6.85, 10% glycerol, 1 mM DTT. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a single major peak
30 at 70 -85 mM sodium phosphate (Table 1a). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 40 -45% of total activity loaded (Table 1a). Fractions containing GS-7340 Ester Hydrolase activity (HAP pool) were pooled, snap frozen in liquid nitrogen and stored at -70°C .

High Resolution Gel Filtration Chromatography:

The BS-HIC pool (5ml / 1.25×10^9 cells) was defrosted, concentrated to 0.05ml using a 5kDa molecular weight cutoff concentrator (20ml Vivaspin concentrator, Viva Science, Carlsbad, CA), and loaded onto a high resolution Gel Filtration column (8mm X 300mm, KW 802.5; Shodex, Thomas Instrument Co., Oceanside, CA), previously equilibrated with 25mM Tris, pH 7.5, 150mM NaCl, 10% glycerol, 20mM CaCl₂, 1mM DTT (KW 802.5 column buffer). Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a single major peak at in fractions corresponding to an apparent molecular weight of 70 – 100 kDa (Table 1a). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was >75% of total activity loaded (Table 1a). Fractions containing GS-7340 Ester Hydrolase activity (KW 802.5 pool) were pooled, snap frozen in liquid nitrogen and stored at –70°C.

Summary of GS-7340 Ester Hydrolase Purification:

The following table summarizes the purification of GS-7340 Ester Hydrolase achieved. Protein was measured by a Coomassie Blue stain colorimetric assay (Bradford Protein Assay, BioRad, Hercules, CA). The Specific Activity (pmoles Metabolite X produced / minute / μ g protein) of the partially purified GS-7340 Ester Hydrolase varied from 666 to 1500. This represents a 222 – 750 fold purification from the PBMC extracts. Overall Recovery of GS-7340 Ester Hydrolase from PBMC extracts was approximately 10%.

Table 1c: Purification Summary of GS-7340 Ester Hydrolase:

| Sample name | PBMC | Protein concentration (mg/ml) | Volume (ml) | Protein (mg) | Total Activity (pmol/min) | Specific Activity pmol/min/ μ g | % Recovery |
|-------------|----------------------|-------------------------------|-------------|--------------|------------------------------|-------------------------------------|------------|
| P0 PBMC | 30 X 10 ⁹ | 5.0 | 200 | 1000 | 2.0 – 3.0 X 10 ⁶ | 2.0 – 3.0 | |
| Q15 Pool | | 0.116 – 0.167 | 300 | 35 – 50 | 1.0 – 1.5 X 10 ⁶ | 20 – 42 | ~50 |
| BS-HIC Pool | | 0.02 – 0.035 | 100 | 2.0 – 3.5 | 0.5 – 0.75 X 10 ⁶ | 142– 375 | ~50 |
| HAP Pool | | 0.02 – 0.03 | 10 | 0.2 – 0.3 | 0.2 – 0.3 x 10 ⁶ | 666 – 1500 | ~40 |
| | | | | | | % Total Recovery | ~10 |

Biochemical Characterization of GS-7340 Ester Hydrolase:

5

Determination of the Isoelectric point (pI) of GS-7340 Ester Hydrolase:

The isoelectric point (pI) of a protein is defined as the pH at which the protein has no net ionic charge. Chromatofocusing is a chromatographic procedure in which a negatively charged protein is bound to a hydrophilic column with a net positive ionic charge. The protein is loaded at a pH 1 to 2 pH units higher than its estimated pI, and the bound protein is eluted by generating a decreasing pH gradient using a pH 3.0 to 4.0 buffer. The proteins will be eluted at a pH corresponding to pI.

An aliquot of the BS HIC pool (20 ml, 5 X 10⁹ cells) was concentrated to 4.0 ml and prepared for chromatofocusing chromatography by exchanging buffer using a desalting column. 1.0 ml aliquots of the concentrated BS HIC pool were loaded onto a 5.0 ml desalting column (5.0 ml HiTrap, Amersham Biosciences, Piscataway, NJ) previously equilibrated with 25mM ethanolamine, pH 7.8 (pH'd with iminodiacetic acid), 10% glycerol (Mono P Buffer A). The desalted GS-7340 Ester Hydrolase activity was loaded onto a chromatofocusing column (5mm X 5mm HR Mono P, Amersham Biosciences, Piscataway, NJ) previously equilibrated with Mono P Buffer A. Bound protein was eluted with a 20CV gradient to pH 3.6 with 10 ml /

100 ml Polybuffer 74 (Amersham Biosciences) pH'd to 4.0 with iminodiacetic acid. This chromatofocusing protocol produces a linear pH gradient from pH 7.8 to pH 3.6. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a
5 single major peak at pH 5.5 to 4.5. Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 65 -70% of total activity loaded. Fractions containing GS-7340 Ester Hydrolase activity (KW 802.5 pool) were pooled, snap frozen in liquid nitrogen and stored at -70°C.

10 **Inhibition of GS-7340 Ester Hydrolases by Serine Hydrolase Inhibitors:**

Fluorophosphonate / fluorophosphate (Diisopropylfluorophosphate (DFP)) derivatives, isocoumarins such as 3,4 dichloroisocoumarin (3,4-DCI) and peptide carboxyl esters of chloro- and fluoro-methyl ketones (AlaAlaProAla-CMK, AlaAlaProVal-CMK, PheAla-FMK) are known effective inhibitors of serine hydrolases (Powers and Harper 1986; Delbaere and
15 Brayer, 1985; Bullock et al 1996; Yongsheng et al 1999; Kam et al 1993). Inhibition of the enzymatic production of metabolite X from GS-7340 was monitored using the following Ester Hydrolase Inhibition assay: Varying amounts of partially purified GS-7340 Ester Hydrolase and control enzymes (human leukocyte elastase (huLE), porcine liver carboxylesterase (PLCE)) were incubated with [¹⁴C] GS-7340 in the presence and absence of varying amounts
20 of known serine hydrolase inhibitors at 37°C for 10 – 90 min. The production of [¹⁴C] Metabolite X was monitored by measuring the amount of radioactivity retained on an anion exchange resin (DE-81). The final reaction conditions were: 25 mM 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.5, 100mM NaCl, 1 mM DTT, 30 μM [¹⁴C] GS-7340, 0.1% NP40 varying amounts of enzyme and inhibitors (1.0μM – 1mM) in a final volume
25 of 60 μl. The reaction mixture was incubated at 37°C and at 10, 30 and 90 minutes, 17μl of the reaction mixture was spotted onto a DE-81 filter. The filter was processed and the amount of [¹⁴C]-Metabolite X present was determined as described above. Activity was expressed as pmoles Metabolite X produced / minute / volume enzyme sample. Inhibition of Ester Hydrolase and control hydrolases was expressed as percent activity present at a given
30 concentration of inhibitor compared to hydrolase activity in the absence of the inhibitor. The results of the inhibition experiments are shown in Table 2A/B. The serine hydrolase inhibitors, 3,4-DCI and DFP inhibit GS-7340 Ester Hydrolase with estimated IC₅₀'s of 4.0 and 30 μM,

respectively. The peptide chloro- and fluoro-methyl ketones are less effective inhibitors with estimated IC₅₀'s of 100 –400 μ M (Table 2 A / B).

Table 2A: Inhibition of GS-7340 Ester Hydrolase and Control Enzymes by Serine Hydrolase Inhibitors

| Inhibitor | IC50 (μM) | | |
|-----------------------------------|-------------------------------|-------|------|
| | GS-7340 Ester Hydrolase | PLCE | huLE |
| 3,4-dichloroisocoumarin | 4.0 | 250 | 3.0 |
| MeOSuC-Ala-Ala-Pro-Ala-CMK | 200-400 | >1000 | 60 |
| MeOSuc-Ala-Ala-Pro-Val-CMK | 100 | >1000 | 4.0 |
| Biotin-Phe-Ala-FMK | 100 | >1000 | 100 |
| DFP | 30 | 0.05 | - |

5 Table 2B: Inhibition of GS-7340 Ester Hydrolase and Control Enzymes by Serine Hydrolase Inhibitors

| | Inhibitor (μM) | Relative Activity (%) | IC50 (μM) |
|--------------------------------|-------------------|--------------------------|--------------|
| GS-7340 Ester Hydrolase | | | |
| 3,4-dichloroisocoumarin | 1.0 | 100 | 4.0 |
| | 10 | 25 | |
| | 100 | 5 | |
| | 1000 | <2 | |
| DFP | 1.0 | 100 | 30-40 |
| | 10 | 90 | |
| | 100 | 35 | |
| | 1000 | <2 | |
| Biotin-Phe-Ala-FMK | 1.0 | 100 | 100 |
| | 10 | 95 | |
| | 100 | 50 | |
| | 1000 | <2 | |
| PLCE | | | |
| 3,4-dichloroisocoumarin | 1.0 | 100 | 250 |
| | 10 | 100 | |
| | 100 | 90 | |
| | 1000 | 20 | |
| DFP | 0.001 | 100 | 0.05 |
| | 0.01 | 90 | |
| | 0.1 | 20 | |
| | 1.0 | <2 | |
| Biotin-Phe-Ala-FMK | 1.0 | 100 | >1000 |
| | 10 | 100 | |
| | 100 | 100 | |

| | | | |
|--------------------------------|------|-----|-----|
| | 1000 | 80 | |
| huLE | | | |
| 3,4-dichloroisocoumarin | 1.0 | 100 | 4.0 |
| | 10 | 25 | |
| | 100 | 5 | |
| | 1000 | <2 | |
| Biotin-Phe-Ala-FMK | 1.0 | 100 | 100 |
| | 10 | 93 | |
| | 100 | 48 | |
| | 1000 | <2 | |

Summary of Biochemical Characterization of GS-7340 Ester Hydrolase:

- Summarizing, GS-7340 Ester Hydrolase is a novel enzyme characterized by being capable
- 5 of being recovered from human PBMCs by a process comprising
- (a) lysing human PBMCs;
 - (b) extracting the lysed cells with detergent;
 - (c) separating the solids from supernatant and recovering the supernatant;
 - (d) contacting the supernatant with an anion exchange medium;
 - 10 (e) eluting the Hydrolase from the anion exchange medium;
 - (f) contacting the eluate with a hydrophobic chromatographic medium; and
 - (g) eluting the Hydrolase from the hydrophobic chromatographic medium.

GS-7340 Ester Hydrolase is useful in screening candidate compounds to assess the

15 likelihood that they can be processed to form depot metabolites in lymphoid tissue. The candidates are assayed in the same fashion as described herein for GS-7340, taking into account differences in the nature of the suspected substrate as will be apparent to the ordinary artisan.

20 GS-7340 Ester Hydrolase optionally is labelled with a detectable group such as a radiolabel or covalently bound to an insoluble matrix such as Sepharose using techniques heretofore employed for other enzymes having similar properties, as will be apparent to the ordinary artisan.

25 GS-7340 Ester Hydrolase has the following properties:

- 1) GS-7340 Ester Hydrolase can be partially purified from fresh PBMC Extracts: SA = 666 -1500 pmoles MetX/min/ug protein.

2) GS-7340 Ester Hydrolase can be separated from non-specific Esterases capable of cleaving alpha-naphthyl acetate (ANA), a non-specific substrate shown to be cleaved by many carboxylesterases and hydrolases.

5

3) Multiple GS-7340 Ester Hydrolase activity peaks are not eluted from columns during purification.

4) The MW of GS-7340 Ester Hydrolase on Gel Filtration is ~ 70 - 100kDa

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5) The pI of GS-7340 Ester Hydrolase is pH 4.5 -5.5

6) Evidence to date suggests that the SA of isolated GS-7340 Ester Hydrolase is likely to be > 10,000.

15

7) The serine hydrolase inhibitors, 3,4-DCI and DFP inhibit GS-7340 Ester Hydrolase with estimated IC₅₀'s of 4.0 and 30 μ M, respectively. The peptide chloro- and fluoro-methyl ketones are less effective inhibitors with estimated IC₅₀'s of 100 – 400 μ M (Table 2 A / B).

20

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Example: Candidate Compounds

A large number of examples describing the preparation of candidate compounds active against HIV protease, HIV integrase and HIV polymerase (non-nucleotide reverse transcriptase inhibitors, or NNRTIs) are found in copending applications and are set forth

25 below. These compounds are examples of candidate compounds that are typical of those which are suitable for use in the method and libraries of this invention.

30

Incorporation by Reference

All publications and patent applications cited herein are incorporated by reference to the same extent as if the full text of each individual publication or patent application was contained herein. The incorporated text will be apparent from context if not specifically set forth. Incorporated by reference are (a) US patent applications 60/373,533 and 60/375,665

(attorney docket 257.P and 257.P2) and the section 111(a) application filed of even date hereof based on such applications and (b) U.S. patent application 60/375,622 (attorney docket 260.P) and the section 111(a) application filed of even date hereof based on such application.

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